TRACKING SPATIOTEMPORAL MOVEMENTS OF DUNLIN (CALIDRIS ALPINA ARTICOLA) MIGRATION THROUGH STABLE ISOTOPE ANALYSIS

by

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Tracking Spatiotemporal Movements of Dunlin (*Calidris alpina arcticola*) Migration Through Stable Isotope Analysis.

Thesis directed by Assistant Professor Michael Wunder.

ABSTRACT

This thesis describes my investigations into the migratory and breeding behaviors of Dunlin (*Calidris alpina arcticola*) using chemical signals contained within the tissues of these long-distance migratory shorebirds. The ratios of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in the tissues of these birds serve as an intrinsic record of past dietary consumption. By studying the variation of these ratios over time and between tissues, I was able to make inferences about past behaviors.

Chapter I describes how I exploited the change that occurs in stable carbon isotope values in blood and muscle tissues after the spring migration to estimate individual arrival dates on the breeding grounds. Doing so requires an accurate isotope turnover rate, so I evaluated an existing experimentally-determined rate and a theoretically-derived rate against an *in-situ* turnover rate I calculated using a novel recapture procedure developed for this study. Comparing the arrival date estimates obtained using these three turnover rates to onsite conditions and to earliest possible arrival dates determined in a subset of the sampled birds tagged with geolocation devices allowed me to evaluate their efficacies.

Chapter II expands upon the findings of the first chapter by using the arrival date estimates based on the *in-situ* turnover rate to improve our understanding of Dunlin behavior on the breeding grounds. I also present the stable isotope values of feather

tissues which provided additional information about the isotope niches of Dunlin at different times and places. Due to their differing molt schedules, breast feathers contain isotope values reflective of the diet in the non-breeding season while the isotope values primary feathers are reflective of the diet on the breeding grounds. The variation within and between the isotope values of these two feather types provides useful insight about the distribution and behavior of Dunlin during the winter-through-summer portion of their annual cycle.

The form and content of this abstract are approved. I recommend its publication.

Approved: Michael Wunder

DEDICATION

I dedicate this work to my wife, Nola Miguel, and my son, Leopold Doll. Without their support and patience, I would not have been able to complete this work. I love them dearly.

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I thank my committee members for their advice and encouragement along the way: Mike Greene for the subtle, yet needed, redirections in focus and emphasis which have clearly strengthened this work; Craig Stricker for facilitating the physical isotope analyses as well as for providing essential knowledge to my comprehension of why and how isotopes can be used to improve understandings of animal behavior and ecological processes; Rick Lanctot for facilitating all of the field aspects of this research and for sharing his wealth of knowledge about the biology and ecology of the shorebird communities I have been so lucky to have worked with. As my advisor and committee chair, I will single out Mike Wunder to thank him for taking me under his wing and mentoring me through this process. Without his help, direction and redirection this work would not be as strong as it is and I would not be the scientist I have become.

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ISOTOPIC TURNOVER RATES ESTIMATED FROM CAPTIVE FEEDING EXPERIMENTS DO NOT TRANSLATE TO WILD ANIMALS: ESTIMATING ARRIVAL DATES IN ARCTIC-BREEDING DUNLIN (*CALIDRIS ALPINA ARCTICOLA*)

Abstract

The use of stable isotope analysis in animal ecology depends upon an accurate description of isotope dynamics within individuals. The prevailing assumption that results from laboratory experiments will apply to free-living animals remains largely unchallenged. I tested this assumption by using stable carbon isotope measurements of blood tissues from migratory Dunlin (*Calidris alpina arcticola*) to estimate individual diet-switch dates associated with arrival on the Arctic breeding grounds.

To estimate arrival times, I used an exponential decay model describing the incorporation of stable isotopes in metabolically active tissue which requires a tissue-specific rate of isotope turnover. The stable carbon isotope ($\delta^{13}C$) turnover rate in Dunlin blood has been experimentally-determined in a captive feeding trial. An existing allometric model relating $\delta^{13}C$ turnover rates to body mass provides a second estimate of this turnover rate in Dunlin. I present a third method for determining isotope turnover rates using a field-based approach in wild Dunlin which have recently completed spring migration.

The results indicate a higher $\delta^{13}C$ turnover rate in free-living birds as compared to rates measured in captive Dunlin and derived from the allometric model. Estimated arrival dates calculated from both the experimental and allometric turnover rates were

substantially earlier than arrival dates calculated from the field-based turnover rate. Evaluating these dates in comparison to environmental conditions at the study site (i.e. snowmelt) and known movements of individual birds based on light-level geolocation suggests that the field-based method yields a more reliable carbon isotope turnover rate.

I propose the faster isotopic turnover rates measured in wild individuals are due to increased metabolic activity resulting mainly from post-migration tissue repair, reproduction and molt. Because experimental conditions may not fully represent the challenges experienced by free-living individuals, I advocate field-based methods for assessing experimentally-determined isotopic parameters prior to their application.

This study presents a novel method for accurately determining individual arrival dates for species that experience a significant isotopic diet-switch without the need for extrinsic tracking devices. As information on individual migration behavior is lacking for most species, this isotopic approach holds great potential for improving our understanding of population dynamics in migratory species.

Introduction

The application of stable isotope analysis has allowed ecologists to examine previously intractable aspects of animal ecology such as connectivity patterns in migratory species and trophic linkages within food webs (Chamberlain *et al*. 1997, Newsome *et al*. 2007). Recent work suggests that stable isotope models can also be used to determine when animals switch from one diet to another (hereafter referred to as 'diet-switch'), which often occur when animals migrate between different habitats (Dalerum & Angerbjörn 2005, Phillips & Eldridge 2006, Oppel & Powell 2010). These isotope

techniques represent a significant addition to existing extrinsic marking methods (banding, UHF radio, GPS; Gauthreaux 1996) for tracking the spatiotemporal movements of migratory animals by providing a method for determining arrival times of migratory individuals that have not been previously captured and marked. As an intrinsic marker technique, stable isotope analysis also avoids some of the limitations of extrinsic markers such as size requirements for animals that can carry the tags, behavior modification, and reduced inferential scope from the larger population to that of marked animals (Morales *et al*. 2010, Robinson *et al*. 2010, and Bridge *et al*. 2011). However, isotope dynamic models require accurate species- and tissue-specific parameters to accurately assess movement patterns.

The idea that stable isotopes can be used to determine diet-switch times is based on the process of tissue turnover during metabolism (Pellettieri & Sánchez Alvarado 2007), where whole cells and cellular components are degraded and replaced. When an animal switches between isotopically distinct diets, turnover leads to an isotopic transition in metabolically active tissues to reflect the new diet. Tieszen, Boutton & Tesdahl (1983) described this transition in a simple time-dependent exponential decay model: $y(t) = y_\infty + (y_0 - y_\infty)e^{-kt}$. In this model, $y(t)$ is the isotope value of a tissue (e.g. blood) t days after a diet-switch. y_0 and y_∞ are the isotopic endpoints of the transition; the isotope values of the tissue at isotopic equilibrium with the old and new diets, respectively. The final parameter, k, is the isotope turnover rate for the tissue in question (units = day^{-1}). Isotopic equilibrium occurs when an animal has fed on an isotopically consistent diet long enough for the tissue to have completely regenerated such that it

reflects the isotope composition of the diet, differing only by the amount of isotopic discrimination (Hobson & Clark 1992a) that occurs during the assimilation process.

Species-specific isotope turnover rates are often determined in captive diet-switch experiments by measuring the change in isotope values of a tissue after manipulating the isotopic composition in an animal's diet (Hobson & Clark 1992b, Evans-Ogden, Hobson & Lank 2004, Bauchinger & McWilliams 2009). Because the tissues sampled for isotopic analysis are usually highly proteinaceous and protein turnover rates scale predictably with body size (Houlihan, Carter & McCarthy 1995), Carleton and Martinez del Rio (2005) proposed a simple allometric model for determining isotope turnover rates of animal tissue. Oppel and Powell (2010) applied this allometric model with the Tieszen *et al*. (1983) isotopic transition model to determine the timing of an isotopic diet-switch in King Eiders (*Somateria spectabilis*) that occurs during their spring migration between the marine wintering environment and their terrestrial breeding grounds. However, I know of no studies investigating the relative suitability of applying either laboratory-determined (experimental) or theoretically-derived (allometric) isotope turnover rates in models for wild populations of animals.

Therefore I designed this study to examine isotopic turnover in a wild population of migratory Dunlin (*Calidris alpina*, Linnaeus; Fig. I.1). The *C. a. arcticola* subspecies breeds in the terrestrial tundra environment of Northern Alaska and migrates to wintering areas in the coastal and estuarine environments of Southeast Asia (Warnock & Gill 1996, Lanctot *et al*. 2009). Dunlin migration routes are presumed to be primarily coastal where individuals feed primarily on marine organisms. In contrast, Dunlin breed inland and consume terrestrial organisms found either on or near their breeding territories (Holmes

1966a). Due to the isotopic fractionation that occurs during the exchange of carbon between dissolved bicarbonate and atmospheric CO_2 (the main inorganic carbon sources of marine and terrestrial ecosystems, respectively), marine based $\delta^{13}C$ values are approximately 7‰ greater than corresponding C_3 plant based terrestrial values (Craig 1953, Peterson & Fry 1987) that are common to the tundra ecosystem. Similar differences in $\delta^{15}N$ values between marine and terrestrial environments have been demonstrated (Chisholm *et al*. 1982, Schoeninger & DeNiro 1984, France *et al*. 1998). Thus, I reasoned that upon arrival to the breeding grounds, Dunlin tissues will transition from relatively high marine isotope values characteristic of their winter diets to lower terrestrial isotope values of the diet on their breeding grounds.

Both single- and dual-tissue models have been proposed for estimating diet-switch dates from isotope data (Phillips & Eldridge 2006, Klaassen *et al*. 2010). Because the dual-tissue model exploits the differential between isotopic turnover rates in various tissues, it requires species-specific turnover rates for two tissues with sufficiently different turnover rates and isotope values of each tissues equilibrated to the original diet. In contrast, the single-tissue model requires only one turnover rate and isotope values of the tissue equilibrated to the original diet and to the new diet. A sensitivity analysis on these models has indicated the single-tissue performs more reliably than the dual-tissue models (Klaassen *et al*. 2010). For this reason, and because a $\delta^{13}C$ turnover rate for Dunlin whole blood had previously been experimentally-determined, I chose to explore the single tissue model to estimate diet-switch times .

To determine a $\delta^{13}C$ turnover rate Evans-Ogden *et al*. (2004) exposed captive Dunlin to a simulated marine-terrestrial diet-switch similar to what would be experienced

in the wild. By analyzing blood sampled repeatedly after the diet-switch, they determined the rate of stable carbon isotope turnover in whole blood. They also determined diet-tissue discrimination factors for various Dunlin tissues at equilibrium with the diet; factors I required as a part of my study design. Therefore, this species was an ideal candidate for evaluating differences in the predictive consequences of using experimentally- and theoretically-derived turnover rates as applied to a wild population. Moreover, considering the differences in environmental conditions and behavioral stresses between the captive setting of the diet-switch experiment and the natural environment (e.g. molt, migration, reproduction, weather, resource availability), I used this opportunity to investigate the validity of applying experimentally-derived turnover rates from captive birds.

Here I describe a novel recapture approach to estimate the isotope turnover rate of whole blood in wild-caught Dunlin under naturally occurring conditions. I then estimated diet-switch dates using this *in-situ* turnover rate and compared them to dates determined from experimentally- and theoretically-based (allometric) models. Finally, assuming diet-switch dates equate to arrival dates, I evaluated these three techniques by comparing the estimated dates of arrival to dates of last known locations of a subset of individuals as they moved northward during migration (determined using light-level geolocators, Clark *et al*. 2010) as well as to when local environmental conditions were likely suitable for Dunlin. Understanding the validity of laboratory-derived turnover rate estimates is important for evaluating previous efforts to track animal movements with stable isotopes and will be a vital factor in the design of future isotope studies. In contrast to commonly available population estimates of arrival for migratory species, the

techniques described here demonstrate a simple method for obtaining accurate arrival data at an individual level. This type of individual data is essential to understanding the drivers of population dynamics in migratory species and monitoring the ecological impacts of global climate change.

Methods

Study Site Blood and muscle samples from adult Dunlin were collected in June and July of 2010 and 2011 on the accessible lands within a 25 km radius around the city of Barrow, Alaska (71°17′44″N, 156°45′59″W; Fig. I.1). The habitat is primarily tundra comprised of grasses and sedges, with prostrate willows and flowering herbs occurring on the drier, elevated areas (MacLean & Pitelka 1971). Nesting Dunlin were sampled on six 600 m x 600 m long-term study plots located to the southeast of Barrow (Naves *et al*. 2008) and the area around Fresh Water Lake located southwest of Barrow.

Sample Collection Each year, I obtained muscle tissue from the right pectoralis muscle of ten adult Dunlin lethally collected with an air-powered pellet gun. Five "pre-breeding" individuals were collected upon arrival to the breeding grounds (1 June to 6 June) and five "post-breeding" individuals were collected at the end of the breeding season (20 July to 24 July). I also collected blood from seven of the 10 pre-breeding and seven of the 10 post-breeding birds using a non-heparinized capillary tube. I was unable to obtain sufficient quantities of blood for analysis from the remaining six. Collected specimens were stored frozen for subsequent preparation and analysis.

I also live captured adult Dunlin at nests using bow nets (Bub 1995). Nests were located by systematically searching the study plots and nearby areas (Naves *et al.* 2008).

I captured 103 and 120 adult Dunlin in 2010 and 2011, respectively. Thirty-three and 21 of these individuals in 2010 and 2011, respectively, were captured a second time on their nests (one individual was recaptured after initiating a second nest). Time between capture events ranged from 9 to 26 days. Adults were uniquely banded with US Geological Survey (USGS) metal bands, color bands and alpha-engraved flags. In 2010, I equipped 51 adults with light-level geolocators affixed to leg-bands (Clark *et al*. 2010); 14 were subsequently retrieved in working condition in 2011. Whole blood samples (140-210 μl) were collected from the brachial vein of the wing using non-heparinized capillary tubes and blown onto clean glass microscope slides to air-dry. The sample was later scraped into Eppendorf tubes, sealed and stored at room temperature (S.D. Newsome, personal communications). Adults were sexed using discriminant function equations derived for this subspecies or with conventional molecular techniques (Griffiths *et al*. 1998, Gates 2011).

All trapping, handling and collection procedures were carried out in accordance with the University of Colorado Denver Institutional Animal Care and Use Committee protocols (92010(05)1C, 92010(05)1E) and under USFWS (MB085371-14), State of Alaska Department of Fish and Game (10-044, 10-130, 11-018, 11-131) and North Slope Borough Planning and Community Services (10-310, 11-347) permits. Following each field season, I transported all samples to the University of Colorado Denver for storage and tissue preparation. Subsequent sample preparation and stable isotope analysis was conducted in the laboratories of the USGS.

Isotopic Analysis Muscle tissues were lyophilized and homogenized followed by lipid extraction prior to analysis of 2 mg (±0.05) aliquots in tin capsules. The lipid extraction procedure is similar to that described in Stegall *et al*. (2008) using a soxhlet apparatus with a heated azeotropic solvent solution of two parts chloroform to one part methanol. Dried whole blood samples were used in their field-stored form and weighed in 1 mg (±0.05) aliquots into tin capsules. Prepared samples were analyzed using an elemental analyzer (Carlo Erba) interfaced to a Micromass Optima mass spectrometer (Fry *et al*. 1992). Isotopic results are reported in per mil (‰) using standard δ notation as described in Sulzman (2007).

Isotopic data were normalized to V-PDB, and air using the primary standards USGS 40 (-26.24‰ and -4.52‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively) and USGS 41 (37.76‰ and 47.57‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively). Analytical error was assessed by replicate measures of primary standards (<0.2‰ for both isotopes across all analytical sequences) and quality control was assessed using secondary standards analyzed within individual analytical sequences (<0.2 ‰). Accuracy was assessed using primary standards as unknowns, and was within 0.2‰ for both isotopes. Sample reproducibility, determined via duplicate measurements, was generally better than 0.2‰.

Isotope Dynamics Modeling Due to the unpredictable transition of $\delta^{15}N$ values between sampling events of recaptured individuals (see results), the following isotope dynamics modeling was conducted only on the $\delta^{13}C$ data. $\delta^{15}N$ values are reported for informational purposes.

Transition Endpoints. Muscle tissue has been shown to have a slower turnover rate than blood tissue (Hobson & Clark 1992a, Bauchinger & McWilliams 2009). Thus, I assumed muscle tissue from pre-breeding birds would have experienced less turnover since arrival than blood tissue and would therefore more accurately reflect the marine isotope values of the migration diet. I further assumed that muscle tissue of the post-breeding birds would have reached isotopic equilibrium with the terrestrial diet by the time of collection. For each year, I used the $\delta^{13}C$ values of the muscle tissues sampled during the pre-breeding and post-breeding periods to generate distributions of potential endpoints (y_0 and y_∞) for the transition of $\delta^{13}C$ from marine to terrestrial values in whole blood. Using the mean and standard deviation of each group I generated 5,000 sets of initial and asymptotic isotope signatures (2010: $y_0 \sim \mathcal{N}$(-17.8, 1.4), $y_\infty \sim \mathcal{N}$(-25.8, 0.8); 2011: $y_0 \sim \mathcal{N}$(-18.9, 0.9); $y_\infty \sim \mathcal{N}$(-26.5, 1.2)). Evans-Ogden *et al*. (2004) reported a difference in isotope discrimination from diet to muscle (1.9‰) and from diet to whole blood (1.3‰), thus I subtracted 0.6‰ from each muscle $\delta^{13}C$ value to better represent "blood-like" isotope values.

Isotopic Turnover Rates. To derive an experimental turnover rate (k_e) of $\delta^{13}C$ in Dunlin blood, I bootstrapped the mean stable carbon isotope half-life reported in Evans-Ogden *et al.* (2004). I first simulated 5,000 half-life values based on a normal distribution with a mean of 11.2 days and a standard error of 0.8. I then converted these values into turnover rates using the equation k = ln (2) / (half-life). I determined the allometric turnover rate (k_a) using the mass of each captured individual (m_b) and the allometric model from Carleton and Martinez del Rio (2005): $\log_{10}(k_a) = -0.52-0.35*\log_{10}(m_b)$. Because

turnover rates can only range from 0 to 1, I calculated mean turnover rates and 95% confidence intervals using a beta distribution.

I determined the *in-situ* turnover rate (k_i) using the blood isotope values from 54 individuals sampled at two points in time during the incubation period. The isotope values measured at the first and second captures can be described by the equations:

$$y(t_1) = y_\infty + (y_0 - y_\infty)e^{-kt_1} \quad (1)$$

$$y(t_2) = y_\infty + (y_0 - y_\infty)e^{-kt_2} \quad (2)$$

where t_1 and t_2 indicate the number of days since diet-switch to the first and second capture, respectively. Knowing that the number of days between capture events (d) is equal to the difference between the t_2 and t_1, I combined equations 1 and 2 and solved for the turnover rate (k; day^{-1}) as:

$$k = \frac{\ln\left(\frac{y(t_1) - y_\infty}{y_0 - y_\infty}\right) - \ln\left(\frac{y(t_2) - y_\infty}{y_0 - y_\infty}\right)}{d} \quad (3)$$

For each individual, this calculation was performed for each of the 5,000 sets of potential endpoints. I fit these estimates of k to a beta distribution to determine a mean value for each bird. Because some of the simulated asymptotic endpoint values were higher than the measured isotope value (an unrealistic situation) these were excluded and the mean k was actually calculated from between 146 and 4,729 estimates (95% CI: 2,775 – 3,348). I then calculated a mean population turnover rate (k_i) by fitting the beta means of individual's turnover rates to the beta distribution.

Diet-Switch Dates. I independently calculated individual diet-switch date estimates (T) using k_e, k_a, and k_i, respectively, by rearranging the decay function to:

$$t = \frac{\ln\left(\frac{y_0 - y_\infty}{y(t) - y_\infty}\right)}{k} \quad (4)$$

Subtracting *t* from the date of capture gave me the respective experimental (T_e), allometric (T_a), and *in-situ* (T_i) diet-switch date estimates. Using the full distribution of potential initial and asymptotic endpoints resulted in a distribution of diet-switch date estimates for each individual. Because these distributions were non-normal (Shapiro-Wilks normality test: $W = 0.85$, $p < 0.001$), I calculated a median diet-switch date estimate for each individual and report variability as median absolute deviation (MAD). For recaptured birds, I used only the blood isotope value from each individual's first capture due to the reported lack of reliability in diet-switch date estimates as the tissue approaches the asymptotic value (Oppel & Powell 2010).

Light-Level Geolocation Light-intensity data recorded by geolocators were used to generate migration track lines of 14 individuals captured on the breeding grounds in 2011. I used sunrise and sunset times, indicated by the light-intensity data, to determine day-length and solar midnight which were then used to infer latitude and longitude, respectively. Because the sun does not set north of the Arctic Circle during the end of the spring migration period, I was unable to track individuals above ~66.6° N latitude. Thus, I used the date and location when birds crossed ~66.6° N moving northward to calculate an earliest possible arrival date in Barrow, Alaska, assuming a non-stop flight with an average flight speed of 75 km/hr (Warnock and Gill 1996). The error associated with geolocator estimates varies with several factors (Fudickar, Wikelski & Partecke 2011). Using data from dunlin at known locations of similar latitude and solar season, the 90th percentile of errors are estimated at ~190km (S. Yezerinac, unpublished data). If I made

the conservative assumption that this error was quadrupled at the Arctic Circle, the reported last known location would still be within a half day's flight of actual locations. Due to the equal probability of this error either increasing or decreasing the remaining distance to travel, I chose to ignore this error in my calculations.

Availability of Terrestrial Breeding Areas Terrestrial breeding areas are suitable for Dunlin when snow recedes and birds gain access to invertebrates on the tundra. To evaluate when breeding areas might be suitable each year, I estimated the percent snow cover on 36 quadrats distributed throughout each of the six 36 hectare study plots. Snow cover was estimated using standardized protocols (Arctic Shorebird Demographic Network Protocol Subcommittee 2010) to the nearest 5% and was done every other day until only 10% of the area within plots remained snow covered. Percent snow cover values for days without direct observations were computed by taking the mean of the immediately preceding and following days' values. I excluded data from a plot located at the Barrow landfill because snowmelt occurred earlier as a result of human activities (Saalfeld *et al.* 2012).

Evaluating Arrival Dates I first evaluated the diet-switch date estimates (i.e. my proxy for arrival date) by comparing the average percentage of ground covered with snow with the cumulative number of captured birds present on the breeding grounds as determined by my diet-switch date estimates using a Pearson's product-moment correlation test. I then compared diet-switch date estimates of the 14 birds equipped with light-level geolocators to the earliest possible arrival date as calculated from their last known location south of the Arctic Circle.

Unless otherwise noted, all comparisons between datasets and estimates were performed using a Student's T-test. All analyses were conducted in the R statistical computing package (version 2.15.3; R Development Core Team, 2013).

Results

Muscle Isotope Values The isotopic differences in the muscle tissue between the pre- and post-breeding periods showed a clear decrease in $\delta^{13}C$ and $\delta^{15}N$ values, although $\delta^{15}N$ values between pre- and post-breeding individuals overlapped in 2011 in a few cases (Fig. I.2). Lipid extracted muscle samples from the pre-breeding birds had mean $\delta^{13}C$ values of -17.8 ± 1.4‰ SD (2010) and -18.9 ± 0.9‰ SD (2011). Those from the post-breeding Dunlin had mean $\delta^{13}C$ values of -25.8 ± 0.8‰ SD (2010) and -26.5 ± 1.2‰ SD (2011). The differences in $\delta^{13}C$ values for each group between years were not statistically significant (pre-breeding: $t = 1.4$, $df = 7$, $p = 0.2$; post-breeding: $t = 1.1$, $df = 7$, $p = 0.3$). Pooling both years, the pre-breeding and post-breeding groups were significantly different ($t = 15.0$, $df = 17$, $p < 0.001$).

Lipid extracted muscle samples from the pre-breeding birds had mean $\delta^{15}N$ values of 10.7 ± 0.7‰ SD (2010) and 10.5 ± 1.1‰ SD (2011). The post-breeding muscle samples had mean $\delta^{15}N$ values of 7.6 ± 0.5‰ SD (2010) and 8.6 ± 1.4‰ SD (2011). As with $\delta^{13}C$, the difference in $\delta^{15}N$ values for each group between years was not statistically significant (pre-breeding: $t = 0.2$, $df = 7$, $p = 0.8$; post-breeding: $t = -1.5$, $df = 5$, $p = 0.2$) whereas the difference in both years between the pre-breeding and post-breeding groups was significant ($t = 5.5$, $df = 16$, $p < 0.001$).

For individuals where both blood and muscle tissues were collected, blood was always found to have lower $\delta^{13}C$ isotope values than muscle. In the pre-breeding individuals, the mean difference in $\delta^{13}C$ between blood and muscle was 1.5 ± 1.7‰ SD ($t = -2.2$, $df = 6$, $p = 0.07$). For the post-breeding individuals, the mean difference in $\delta^{13}C$ between blood and muscle was 1.2 ± 0.3‰ SD ($t = -11.7$, $df = 6$, $p < 0.001$). This difference in $\delta^{13}C$ values was twice the value of the muscle-blood discrimination factor derived from Evans-Ogden *et al*. (2004). I chose to use the experimental discrimination value in my modeling and not this field-based value due to uncertainty in the isotope values of dietary items. Variability in dietary isotope values within the terrestrial environment may have greater influence on one tissue over the other because of the difference in timespan of dietary incorporation between blood and muscle tissue. For this reason, inter-tissue discrimination factors are best determined with a controlled, isotopically homogeneous diet. $\delta^{15}N$ blood and muscle values of the pre-breeding birds differed by 0.4 ± 0.2‰ ($t = -4.6$, $df = 6$, $p = 0.004$); however, the difference in $\delta^{15}N$ between blood and muscle of the post-breeding birds was not statistically significant, differing by only 0.04 ± 0.4 ($t = -0.3$, $df = 6$, $p = 0.8$).

Distributions of simulated $\delta^{13}C$ initial endpoint values (y_0) corrected to blood-like values ranged from -24.7 to -13.3‰ in 2010 and from -23.0 to -16.1‰ in 2011. Distributions of $\delta^{13}C$ asymptotic endpoint values (y_∞) corrected to blood-like values ranged from -29.3 to -23.6‰ in 2010 and from -31.4 to -22.9‰ in 2011.

Blood Isotope Values The blood $\delta^{13}C$ values of captured birds decreased throughout the season in both years; however, $\delta^{15}N$ values remained highly variable (Fig. I.3). For

individuals captured twice in 2010, $\delta^{13}C$ values at their first capture ranged from -27.4 to -21.1‰ and at their second capture from -28.0 to -25.1‰ (Fig. I.4a). The mean difference between individual $\delta^{13}C$ values at the first and second captures was 1.6‰ (±1.1SD). In 2011, $\delta^{13}C$ values of individuals sampled at their first capture ranged from -25.6 to -21.1‰ and at their second capture from -27.9 to -25.7‰ (Fig. I.4b). The mean difference between individual $\delta^{13}C$ values at the first and second captures was 2.5‰ (±1.5SD), which is statistically higher than in 2010 ($t = -2.3$, $df = 33$, $p = 0.03$).

In contrast to carbon, $\delta^{15}N$ values at the second capture were not systematically lower relative to the values at the first capture. In 2010, $\delta^{15}N$ values for individuals sampled at their first and second captures ranged from 6.6 to 9.1‰ (Fig. I.4c). In 2011, $\delta^{15}N$ values for individuals sampled at their first and second captures ranged from 5.7 to 9.8‰ (Fig. I.4d). The mean difference between individual $\delta^{15}N$ values at the first and second captures was 0.4‰ (±0.8SD) and did not differ between years ($t = -0.3$, $df = 34$, $p = 0.8$).

For one individual in 2010 the difference in $\delta^{13}C$ values measured between captures was 0.1‰ which was below measurement error. Such a small amount of change over 11 days between captures indicated this individual was already at or near isotopic equilibrium with the terrestrial diet at the time of the first capture. For this reason, I excluded this individual from the calculation of *in-situ* turnover rate.

Isotopic Turnover Rates The whole blood $\delta^{13}C$ turnover rates determined experimentally and based on allometric theory were lower than the turnover rate determined *in-situ* from the captured Dunlin. The data simulated from the reported stable

carbon half-life of 11.2 days derived from Evans-Ogden *et al*. (2004) indicated a beta mean of 0.0665 for k_e (95% CI: 0.0313 - 0.1137). Based on the masses of Dunlin captured in this study (mean 58.6 g), the beta mean k_a was 0.0730 (95% CI: 0.0695 - 0.07663).

The beta mean *in-situ* turnover rate of males (0.0882; 95% CI: 0.0420 - 0.1490) was lower than that of the females (0.1007; 95% CI: 0.0531 - 0.1613), but this difference was not statistically significant (t = -1.6, df = 50, p = 0.11) and therefore the sexes were pooled to determine k_i. Similarly, the beta mean turnover rate in the 2010 population (0.0957; 95% CI: 0.0506 - 0.1530) was not statistically different from the 2011 beta mean turnover rate (0.0919; 95% CI: 0.0413 - 0.1599; t = 0.5, df = 38, p = 0.6), thus I also pooled individual turnover rates for both years resulting in a k_i of 0.0941 (95% CI: 0.0470 to 0.1553).

Diet-Switch Date Estimates The *in-situ* turnover rate consistently indicated a later arrival date than estimates made using either the experimental or the allometric turnover rate (Fig. I.5). Using k_e, the median T_e was May 30 (MAD = 7.4 d, n = 103) and June 4 (MAD = 4.4 d, n = 120) for 2010 and 2011, respectively. Using k_a the median T_a was June 1 (MAD = 7.4 days, n=103) and June 5 (MAD = 4.4 days, n = 120) for 2010 and 2011, respectively. Applying k_i determined in this study, the median T_i was June 7 (MAD = 5.9 days, n = 103) and June 9 (MAD = 4.4 days, n = 120) for 2010 and 2011, respectively.

Evaluating Arrival Date Estimates In 2010, snow surveys indicated that melting on the study plots began after June 3; however, a late blizzard on June 7 contributed to delaying

the snowmelt (Fig. I.5). Study plots were essentially snow-free by June 18th. The cumulative numbers of estimated arrival dates were negatively correlated with the progression of snowmelt in the study areas (Table I.1). In 2010, the strongest correlation between arrival date estimates and snowmelt was found using the *in-situ* method. Approximately 29% of Dunlin arrival dates estimated with the *in-situ* method occurred before June 4th. Significantly larger proportions of the sampled Dunlin (65% and 72%) were estimated to have arrived prior to June 4th using the experimental and allometric methods, respectively (binomial tests: $p < 0.001$). In 2011, substantial melting had occurred prior to the beginning of this study and most Dunlin were estimated to have arrived after the tundra was open. In this year, the correlation coefficients for all methods were approximately equal with values approaching -1.

During the 2011 spring migration, last known locations of individuals with geolocators occurred in eastern Siberia (Fig. I.1). Individuals departed northwards from these locations on dates ranging from May 26 to June 5, thereafter experiencing 24-hr daylight. The remaining great circle distance from these locations to Barrow, Alaska, ranged from 1362 km to 2136 km. Under the assumption of non-stop flight at 75 km/hr, it would take approximately 1 day (range: 0.76 to 1.19 day) to cover these distances. Thus, I added one day to the date of the last known location to determine the earliest possible date of arrival to the Barrow site. Differences between the earliest possible arrival dates and arrival dates derived from the isotope data were 7.2 ± 3.8 (mean ± SD) days for the *in-situ* method, 1.1 ± 4.2 days for the experimental method, and 2.9 ± 3.9 days for the allometric method (one-way ANOVA, $F = 8.773$, $p = 0.0007$; Fig. I.6). Further, none of the estimated dates of arrival from the *in-situ* method indicated birds

were present on the breeding grounds before it was physically feasible based on conservative extrapolations from the migration track lines. In contrast, the experimental and allometric approaches suggested birds were on-site in four and three instances, respectively, before it was physically possible.

Discussion

Isotopic Transitions The difference in muscle $\delta^{13}C$ between the pre-breeding and post-breeding birds (Fig. I.2) clearly demonstrates an isotopic transition from a marine-based diet to a terrestrial-based diet. This transition can also be seen in the blood samples of the captured birds (Fig. I.3a), with relatively high $\delta^{13}C$ values measured early in the season shifting to lower terrestrial values later in the season.

The marine to terrestrial transition was not as apparent for $\delta^{15}N$. Although there was a slight trend toward decreased values (Fig. I.3b), $\delta^{15}N$ was highly variable throughout the season. Whether this is due to overlap in the marine and terrestrial $\delta^{15}N$ sources or the physiology of nitrogen integration during this post-migration, reproductive and molting season, I cannot say. Regardless, the considerable variability in $\delta^{15}N$ of Dunlin blood prevented me from using nitrogen as an informative intrinsic marker for determining turnover rates or estimating arrival times.

Applying the diet-muscle discrimination factor for carbon (Evans-Ogden *et al.* 2004) to the mean isotope measurements of the post-breeding muscle samples indicated a dietary $\delta^{13}C$ signature of -27.7‰ (±0.8 SD) in 2010 and -28.4‰ (±1.2 SD) in 2011. These values correspond closely with the isotope values of invertebrate prey items (-27.7 ± 2.1‰ SD) sampled by Yohannes *et al.* (2010) and with lipid normalized isotope values

(-26.6 ± 3.5‰ SD) of freshwater invertebrates sampled by Oppel and Powell (2010); both of which were sampled in the same general location as this study. The similarity in these values supports my assumption that muscle tissues of the post-breeding individuals were at or approaching isotopic equilibrium with the terrestrial diet.

Turnover Rate Estimation. The turnover rate of $\delta^{13}C$ in Dunlin whole blood determined from this field study (k_i) is considerably higher than that determined experimentally (k_e) or by allometric theory (k_a). The field-determined k_i equates to a half-life of 7.4 days. This is almost four days shorter than the 11.2 day half-life reported in Evan-Ogden *et al.* (2004) and more than two days shorter than a 9.6 day half-life derived from k_a. This difference substantially reduces time estimates required to reach isotopic equilibrium with the terrestrial diet. Given that after four half-lives the blood will have transitioned to over 90% of the asymptotic value, k_i will shorten the equilibration time by almost 9 days as compared to k_a and more than 15 days when compared to k_e. Studies utilizing experimentally-determined or allometrically-derived isotope turnover rates to assess temporal movements of animals are likely to overestimate the amount of time passed since a diet-switch as occurred. Such inaccuracies are certain to bias any correlations between arrival times and other life history events (e.g. nest initiation).

Physiological differences between males and females could potentially result in different turnover rates between the sexes. Evans-Ogden *et al*. (2004) chose to restrict their study to male Dunlin to avoid the potential for sex related differences to bias their results. My results indicate that $\delta^{13}C$ dynamics in Dunlin appear to operate independently of sex. This is particularly interesting considering that I conducted this

study on breeding Dunlin during the height of their reproductive season when females have higher anabolic costs associated with egg formation (Noble *et al*. 1990, Speake, Murray & Noble 1998). However, an experimental investigation conducted on reproductively active individuals with isotopically regulated dietary inputs would be more suitable for addressing the effects of sexual differences and reproduction on isotope dynamics.

Suitability of Turnover Rate Estimates. The turnover rate estimates generated from wild Dunlin in this study differed substantially from those generated by Evans-Ogden *et al*. (2004) on captive Dunlin. This difference is likely due to differences in catabolic and anabolic requirements. In the Evans-Ogden *et al.* (2004) experimental study, the birds were maintained in captivity for over three months to attain isotopic equilibrium before being subjected to a diet switch. The allometric equation for determining turnover rates is based on experimental determinations of turnover rate, including the Evans-Ogden *et al*. (2004) study. In contrast to these experimental conditions, the birds I trapped in Barrow had recently completed an intense migration of thousands of kilometers. This difference is crucial. The captive birds had long since achieved a stable mass and were healthy, well fed and sheltered when the experiment began. The wild birds were dealing with post-migration tissue rebuilding (Blem 1976, Cherry 1982, Piersma 1998, Piersma and Gill 1998, Landys-Ciannelli *et al.* 2003), feather molt (Warnock and Gill 1996), uncertain food supplies (MacLean & Pitelka 1971, Hodkinson 1995, Danks 2004, Tulp *et al*. 2008), stress from potential predation (Scheuerlein, Van't Hof & Gwinner 2001, Lima 2009), and severe weather conditions (Piersma and Morrison 1994, Piersma *et al.* 2003, Schekkerman *et al*. 2003). The wild population also retained the ability to run and fly to

a far greater extent than the captive animals, resulting in higher metabolic rates (Nagy 1987). The combination of these factors clearly requires a greater investment in metabolic processes. Although the captive birds likely experienced elevated stress levels due to their captivity, Dickens, Earle & Romero (2009) showed that, in wild-caught chukar (*Alectoris chukar*), stress levels began returning to baseline levels after about 9 days in captivity. Therefore, it seems reasonable to conclude that stress-induced increase in metabolism of captive Dunlin is unlikely to equate to the stresses wild birds experience.

Another important difference between the Evans-Ogden *et al*. (2004) study and this *in-situ* study was the time of year each was conducted. The majority of wild individuals in my study were captured in June, during the peak of reproductive activities. The captive experiment took place in early February when the reproductive system is essentially dormant. Therefore the wild birds were investing significant resources and energy into gonad development as well as courting, mating, and nesting activities (Blem 1976, Vezina & Salvante 2010) while the captive birds were not.

Additionally, in switching from a marine to a terrestrial environment, Dunlin transition from a highly saline environment to a relatively low saline environment. Gutierrez *et al.* (2012) showed that birds increased their metabolic rate upon switching diets differing in salinity. This enhanced metabolic rate results from changes in physiology associated with restructuring the salt glands. Wiley *et al*. (2012) proposed the importance of salt loads on the variability of δD in Hawaiian petrels (*Pterodroma*

sandwichensis) and salt loads may play a role in dynamics of carbon and nitrogen isotopes as well.

I expect the combination of these factors will yield significant increases in metabolic activity, and are therefore likely responsible for the observed difference in turnover rates between captive and wild Dunlin. Similar factors must be considered on a per species basis when making decisions about suitability of parameters for use in analyzing stable isotope data.

Reliability of Arrival Date Estimates The 2010 median of *in-situ* diet-switch date estimates (T_i), indicative of Dunlin arrival data on the breeding grounds, was four days later than the median of T_a and seven days later than the median of T_e. This difference was smaller in 2011 with a three day difference from the median of T_a and a four day difference from the median of T_e. On an individual basis, differences in estimated diet-switch dates ranged from 0 to 17 days depending on the estimation method. While the magnitude of differences, both at the population level and the individual level, may seem trivial, these minor differences could have a substantial impact on the biological interpretations made from these data. For example, if I wanted to determine how the plasticity of arrival dates is affecting laying dates and reproductive success in response to recent phenological shifts resulting from climate change (see Dunn and Winkler 2010), a misrepresentation of the lag-time between arrival and laying may downplay the importance of the incremental changes experienced in the environment. As the timing of food availability, a key factor for reproductive success, may only differ by a few days

from year to year, minor errors in arrival date estimates may obscure the relationship between arrival and reproduction.

To evaluate the reliability of the three estimates, I first compared them with the progression of snowmelt on the breeding grounds. The two years of this study were very different in snowmelt rates. In particular, 2010 was useful for evaluating my diet-switch date estimates because the spring thaw occurred very late that year (about 8 days later than 2011). In 2010, many of the diet-switch date estimates occurred before the beginning of snowmelt, regardless of which turnover rate was used. One possible explanation for this is that Dunlin were using the few snow-free areas located in the city of Barrow and along the limited road system where snow is reduced due to wind obstruction and meltoff occurs more quickly. Casual observations of Dunlin also indicate they began arriving as early as May 26 (R.B. Lanctot, unpublished data); thus the fact that the study plots were covered with snow and not open for foraging would not preclude some Dunlin arriving and feeding elsewhere. However, relative to the size of the population ultimately breeding in the area, the number of Dunlin present at this time was likely quite low due to the limited amount of available habitat. The experimental and allometric methods resulted in a relatively high proportion of estimated arrival dates preceding the onset of snowmelt in 2010. In contrast, my *in-situ* method resulted in a much more moderate proportion of individuals estimated to have arrived by this time. Holmes (1966a) reported that the first wave of Dunlin typically arrive in late May, but arrival of the majority of the population varies from year to year and is commonly delayed in years with unfavorable conditions. If this is the case, then the *in-situ* method

of estimating turnover rates leads to arrival date estimates that are more consistent with field observations.

Like the snowmelt data in 2010, migration tracks from light-level geolocation supported the estimated arrival data generated from the *in-situ* method better than the other methods. Perhaps most important is the finding that both the experimental and allometric turnover rates resulted in arrival dates for some individuals that were not possible given the last known location of these birds during migration and a very conservative estimate of when they might arrive on the breeding grounds. In all but one of these cases, the estimated arrival date actually predates the date of the last known location in Siberia. In contrast, all arrival date estimates made with the *in-situ* turnover rate occurred after a realistic date of arrival for each individual. Lag-times between the date of last known location and T_i suggest stopover times of 3 to 15 days during the last stretch of migration through the Arctic. Although inter-individual variation in turnover rate is certain to introduce inaccuracies when applying population mean values at an individual level, the results indicate that the field-based turnover rate more accurately describes the isotope dynamics of free-living individuals than the experimentally- or theoretically-determined rates.

Conclusion

This study provides further support to the theory that stable isotope values measured in blood tissues can be used to estimate arrival times of animals that migrate between isotopically distinct environments. However, I have shown how differences in conditions between laboratory and field settings are likely to alter the isotope dynamics

experienced by individual animals in these environments. This study demonstrates a simple and cost-effective method for determining real-world values of the isotopic parameters required for accurate assessments of the temporal movements of animals. Including distributions of potential isotopic endpoint values allowed me to account for individual variability in dietary consumption. Data from birds tagged with geolocators confirmed that using the *in-situ* isotope turnover rate in my model resulted in more reliable arrival dates than the experimental and allometric turnover rates. Whenever feasible, I would promote the use of field-testing to assess the appropriateness of experimentally-determined parameters.

The novelty of this method is that it provides accurate individual level arrival data without the need for previous handling of the animals or attaching extrinsic monitoring devises. Because migration arrival dates can have notable effects on both individual survival and reproductive success (Both and Visser 2001, Newton 2006), the inclusion of individual-level data into population dynamics models could greatly improve our understanding of the factors driving fluctuations in abundance and demographics. With global climate change and widespread habitat destruction causing dramatic ecological changes which impact species at both the individual and population scales, individual-level data will be essential for developing effective monitoring and conservation strategies to protect the biodiversity of this planet.

Tables and Figures

Table I.1. Correlations between Dunlin arrival estimates and snowmelt

Correlations between cumulative number of Dunlin arrivals estimated overtime and percent snow cover measured in study plots. All correlations were significant with $p < 0.001$. T_e, T_a, and T_i represent the median arrival date estimates of individuals as calculated using the experimental, allometric and *in-situ* turnover rates, respectively.

	Pearson correlation coefficients between estimated arrival dates and percent snow cover		
	Cumulative T_e	Cumulative T_a	Cumulative T_i
2010	-0.66	-0.72	-0.83
2011	-0.97	-0.98	-0.98

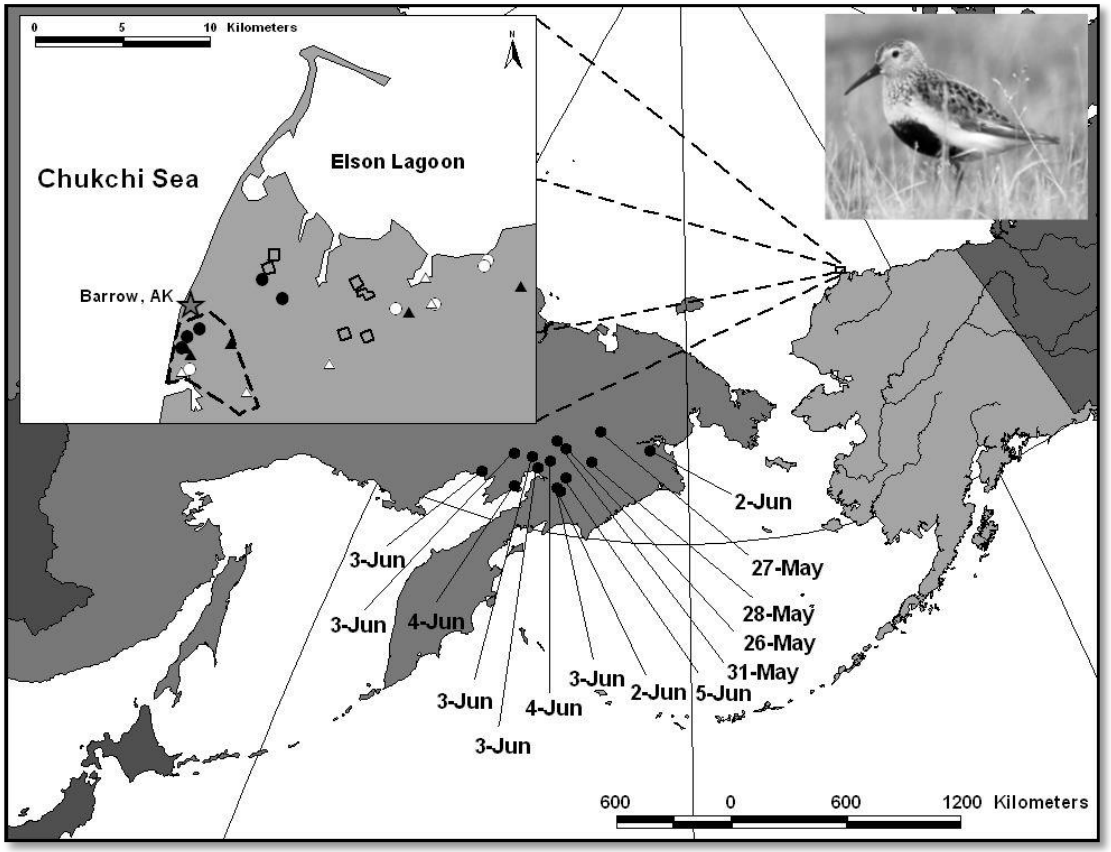

Figure 0.1 Map of Beringia and study site at Barrow, AK.

Map of Beringia displaying the last known location and date when birds crossed northwards into the Arctic for individuals marked with light-level geolocators. Inset right: Photo of Dunlin (*Calidris alpina arcticola*; photo by A. Doll). Inset left: Study area around Barrow, AK and Dunlin sampling locations. The solid line polygons indicate the 6 long-term study plots; the dashed line polygon indicates the Fresh Water Lake study area. Circles and triangles represent locations where Dunlin were collected in 2010 and 2011, respectively (black = pre-breeding, white = post-breeding).

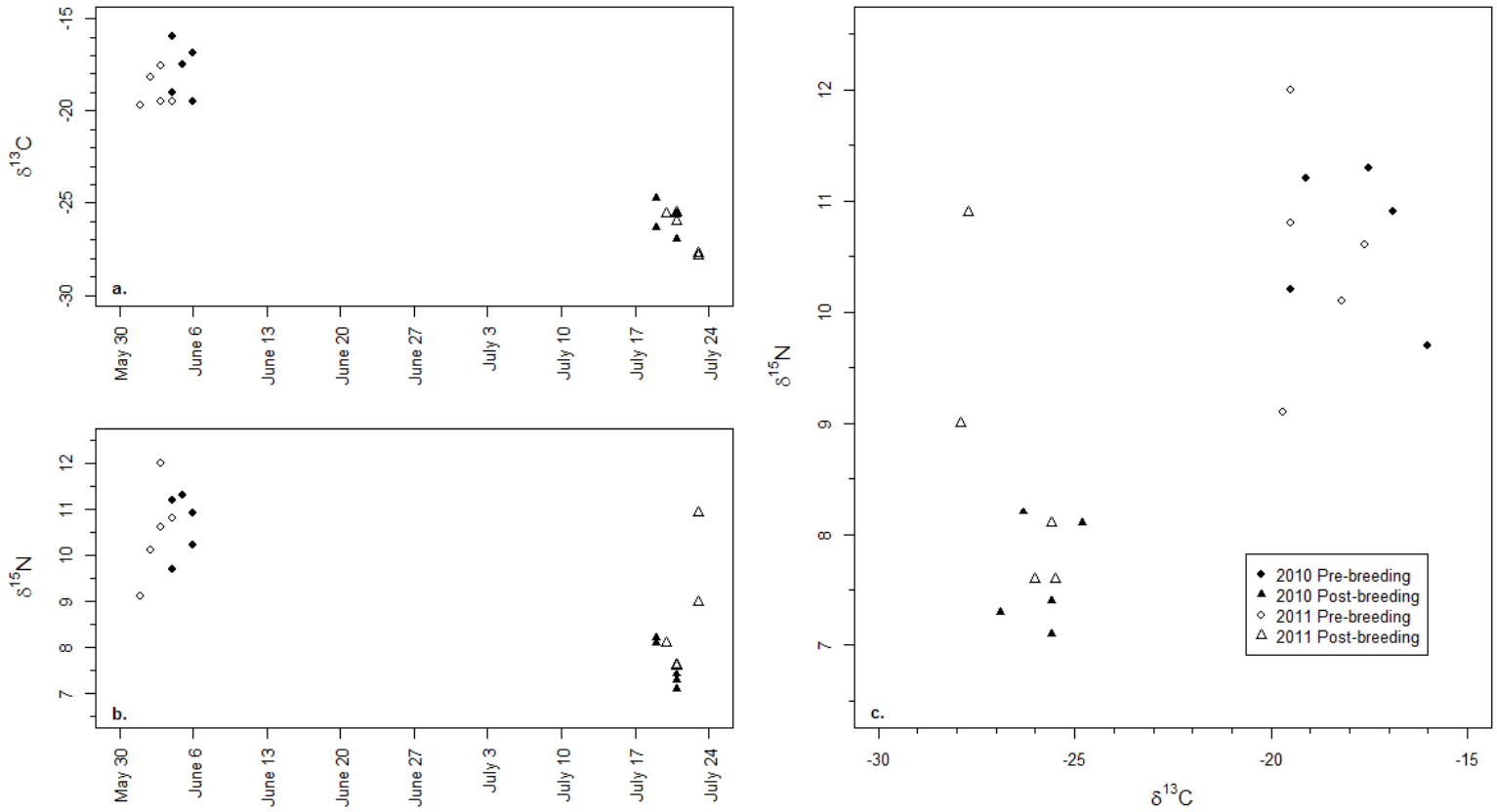

Figure 0.2 $\delta^{13}C$ and $\delta^{15}N$ values of Dunlin muscle tissue.

Plots (**a.**) and (**b.**) display the $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissues, respectively, of collected Dunlin relative to the date of collection. Plot (**c.**) is a biplot of the muscle $\delta^{13}C$ and $\delta^{15}N$ values of the collected Dunlin. Circles represent values of pre-breeding individuals, triangles represent values of post-breeding individuals (solid = 2010, open = 2011).

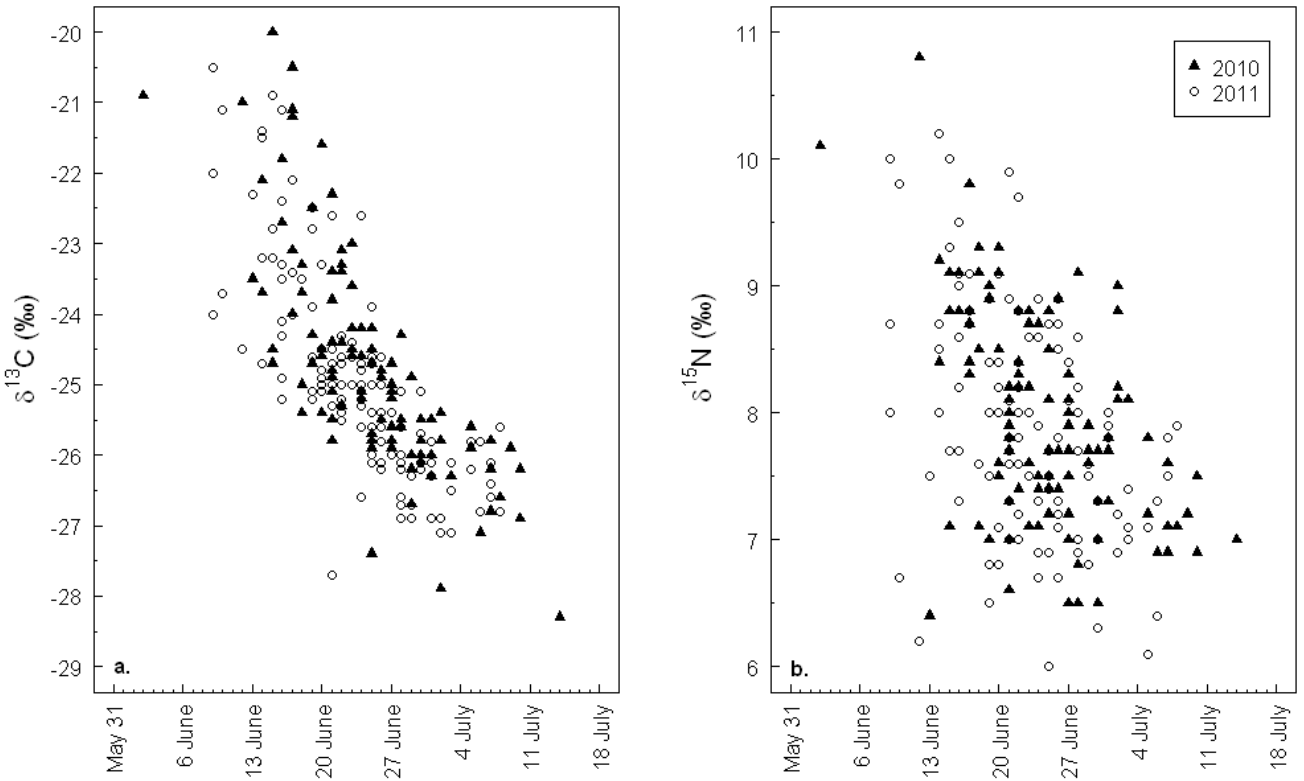

Figure 0.3 Whole blood tissue $\delta^{13}C$ and $\delta^{15}N$ values.

2010 and 2011 $\delta^{13}C$ values (**a.**) and $\delta^{15}N$ values (**b.**) of whole blood tissues relative to the date of capture. For individuals captured twice in a season, only the isotope values from the first capture are included.

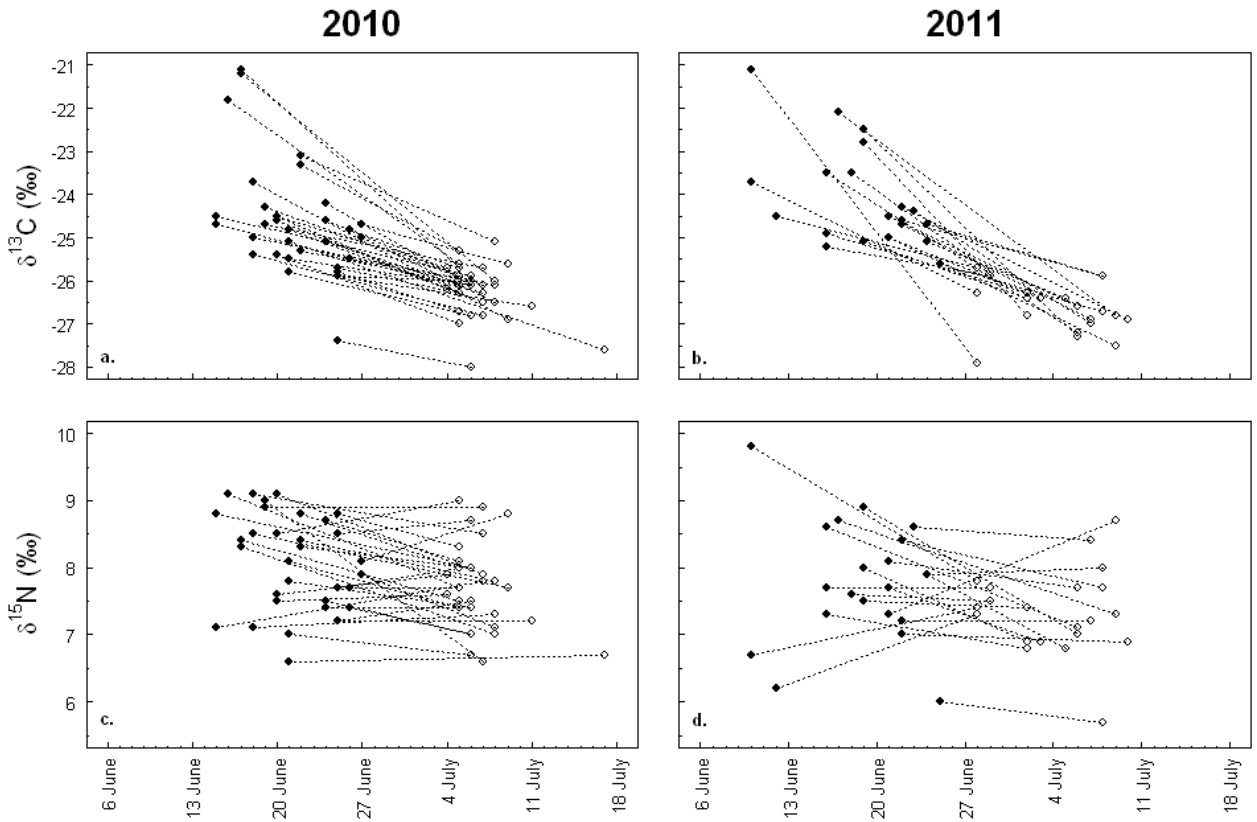

Figure 0.4 Recaptured Dunlin blood tissue $\delta^{13}C$ and $\delta^{15}N$ values.

Blood tissue $\delta^{13}C$ values (**a** and **b**) and $\delta^{15}N$ values (**c** and **d**) from recaptured Dunlin in 2010 (**a** and **c**) and 2011 (**b** and **d**) relative to the date the blood sample was taken. Solid circles represent each individual's first capture; open circles represent their second capture. Dotted lines connect sample points from the same individual.

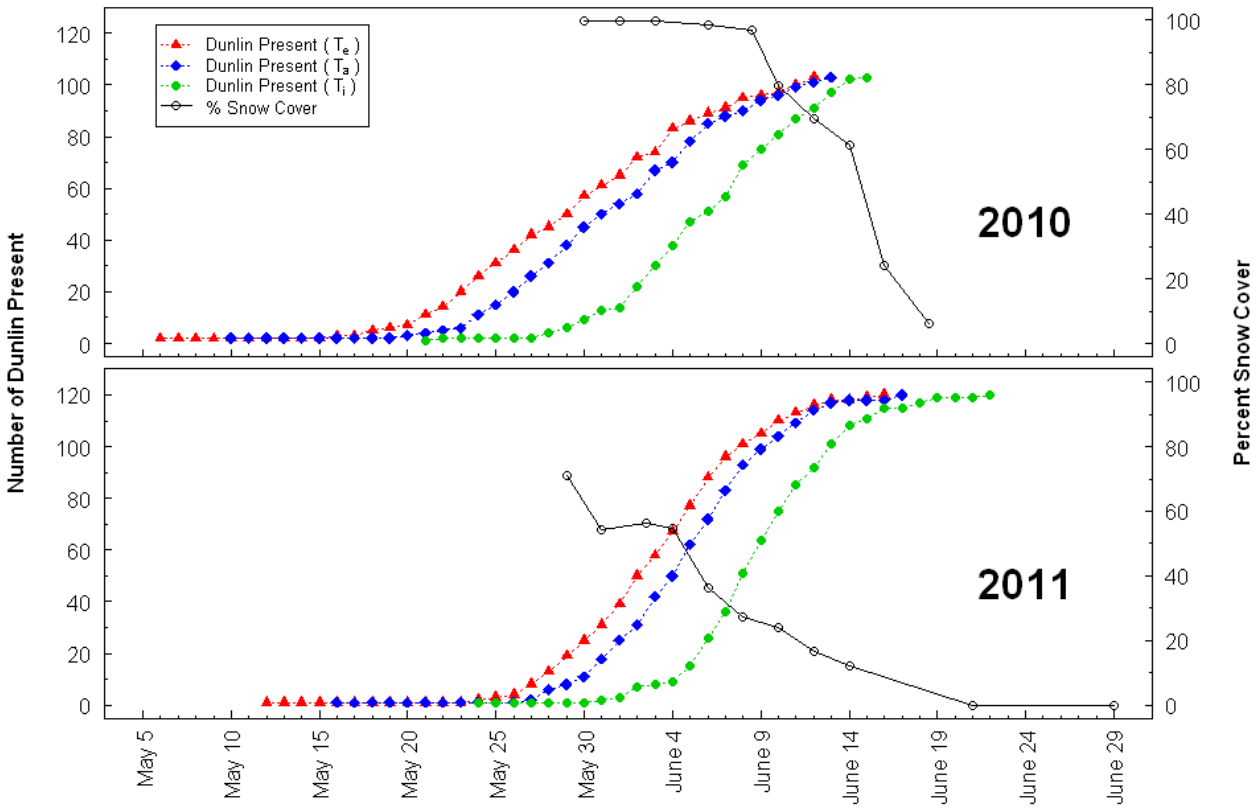

Figure 0.5 Dunlin arrival estimates and snowmelt progression.

Progression of snowmelt and Dunlin arrival estimates for 2010 and 2011. Percent snow cover is represented by the open circles. The solid symbols represent the cumulative number of Dunlin estimated to be present as determined with the experimental (T_e; *triangles*), allometric (T_a; *diamonds*), and *in-situ* (T_i; *circles*) methods.

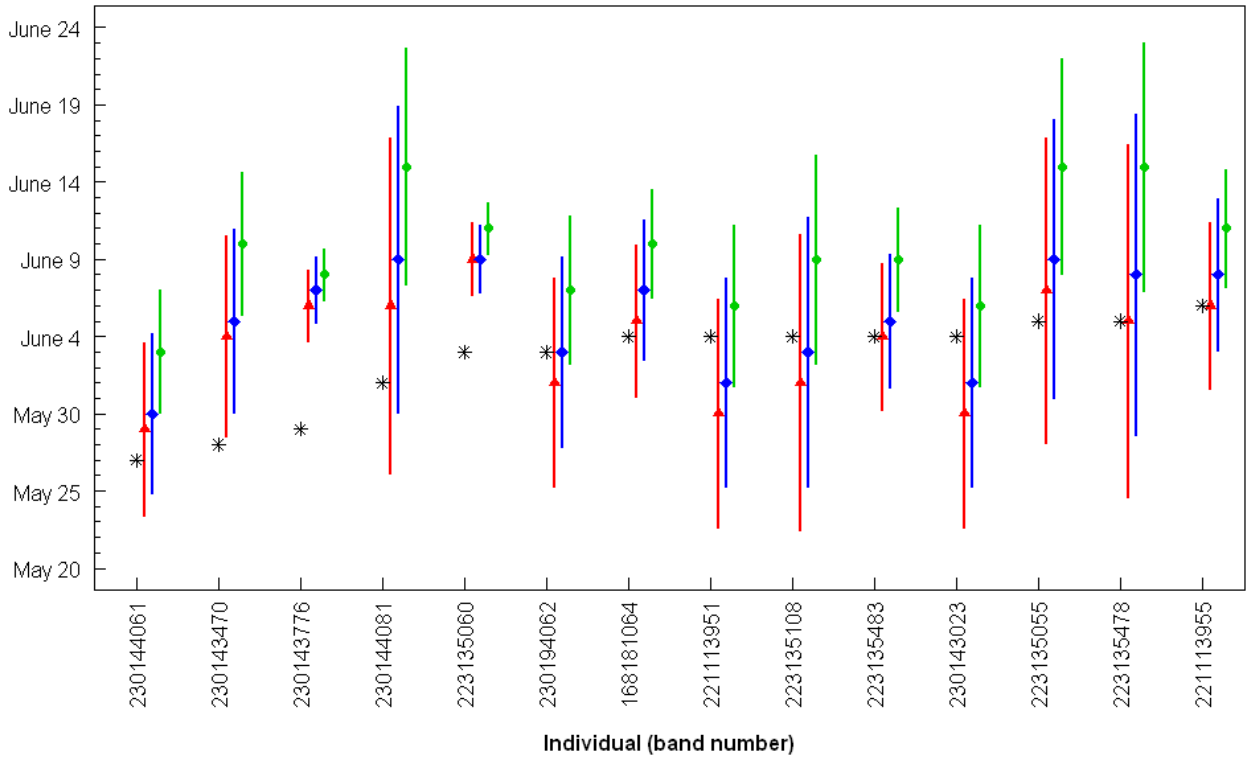

Figure 0.6 Geolocator Dunlin arrival estimates.

Earliest possible arrival dates (asterisks) of birds fitted with geolocators as determined from the date of their last known location during northward migration in 2011. The solid symbols represent the median arrival date estimates determined with the experimental (T_e*; triangles*), allometric (T_a*; diamonds*) and *in-situ* (T_i*; circles*) methods. Error bars represent the median absolute deviation in arrival estimates.

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CHAPTER II

ASSESSING THE RELATIONSHIPS BETWEEN FEATHER ISOTOPE VALUES, POST-MIGRATION ARRIVAL DATES AND NEST INITIATIONS

Abstract

Migratory birds can serve as useful systems for understanding the ecological impacts of changing climate patterns. They often experience exposure to widely diverse habitat types over large geographic scales and annual timescales. The subpopulation of Dunlin (*Calidris alpina arcticola*) that breeds in Northern Alaska is a perfect example of just such a long-distance migrant. Migrating between Alaska and Southeast Asia, these Dunlin are exposed to the perils of breeding the harsh Arctic tundra environment, innumerable potential threats during a migration over thousands of kilometers and the impacts of anthropogenic habitat degradation in their wintering grounds. In this study, I analyze the relationships between isotope values of feathers grown in different stages of the annual cycle, post-migration arrival times (determined in Chapter I), and nesting behaviors. My results demonstrate a clear separation in the isotopic niche space of feathers grown in the breeding and non-breeding areas. The significantly larger niche space of the breast feather isotopes indicates that Dunlin that breed in the localized area around Barrow, AK likely disperse across a much wider geographic distribution during the non-breeding season. A lack of correlation between breast feather isotope values and arrival or nest initiation dates suggests that non-breeding habitat use may not have much influence on behaviors at the breeding grounds. The relatively static nest initiation dates (median: 13 June) between years when weather conditions differed significantly

indicates a targeted optimal initiation date presumably scheduled to optimize hatch date and fledging success. However, the difference between years in lag-time from arrival date to nest initiation suggests that local conditions can significantly alter individual behaviors on the breeding grounds. This study illustrates the utility of combining isotopic techniques with more traditional behavioral information to develop a more comprehensive understanding of individual behaviors. It further demonstrates these methods can be used to monitor the potential to impacts of changes in environmental conditions on population level processes.

Introduction

Recent patterns in global climate change are raising concerns about the ability of wild animals to adapt to rapid changes in the environmental conditions of their ecosystems (Møller *et al.* 2010). Predictions about rising temperatures and changes in precipitation regimes indicate the possibility of drastic alterations to the fundamental factors regulating population dynamics (e.g. food availability). Impacts of this global change are likely to show significant variation in severity across geographic scales (Hurrell & Trenberth 2010). Additional contributions of anthropogenic habitat degradation (pollution, deforestation, overfishing, etc.) are expected to intensify these impacts in many ecosystems. Monitoring the effects of these changes in the biology and ecology of wildlife species will be vital for developing future conservation and management strategies and their effective implementation.

Migratory birds are highly susceptible to changes in climatic conditions due to their seasonal dependence on widely disparate habitats (Sillet, Holmes & Sherry 2000).

The migratory patterns of birds have typically evolved over millions of years as a gradual response to shifting environmental conditions and selective pressures allowing species to optimize their fitness and reproductive success (Bell 2000). For long-distance migrants that utilize Arctic habitats for breeding activities, exposure to multiple environments during migration in various levels of climatic flux only adds to the perils these migrants face in the high latitudes they depend on for reproduction. Unprecedented rates of climatic change (Anisimov *et al.* 2007) are altering Arctic ecosystems in ways these species may not be adapted for. Since 1966, the rate of increase in surface temperatures across the Arctic averages to 0.4°C per decade, with rates exceeding 1 to 2°C per decade in northwestern North America (McBean *et al*. 2005). These increases in temperature are affecting the phenology of the breeding grounds for which the timing of migratory events has been optimized. Temporal advancement of spring thaw is leading to early emergence of the plant and invertebrate species which drive the ecological system (see Both 2010). The ability of birds to adapt their migration schedules to these changing phenologies has been called into question (Both & Visser 2001).

To better understand potential impacts of climate change and habitat degradation on migratory birds, I focused on the Dunlin (*Calidris alpina*); a widely abundant and well-studied shorebird (Warnok & Gill, 1996). This species is threatened not only by changing Arctic conditions but also by widespread habitat degradation in temperate and tropical wintering grounds (Gill & Andres, internal memo, BirdLife International 2012). *Calidris a. arcticola* breeds within the areas of northern Alaska and western Canada experiencing the highest levels of temperature increases. This subspecies has been extensively studied in the breeding grounds with recent work focused on residence time

(Taylor *et al*. 2011), nesting behaviors (Naves *et al*. 2008, Gates 2011), adult and chick survival (Hill 2012) and isotopic dynamics (Chapter I). Recent research in the non-breeding season has provided important information about body condition and population age-structure of Dunlin using the Yellow Sea region of China for wintering and stopover sites (Choi *et al*. 2011). However, much about the biology of *C. a. arcticola* in wintering and migration habits remains unknown.

Wintering distributions of *C. a. arcticola* along the Pacific coast of Asia are believed to include southern Russia, North Korea, South Korea, Japan, Taiwan and China (Warnock and Gill 1996, Lanctot *et al*. 2009). Cao *et al*. (2009) identified substantial numbers of Dunlin wintering along the eastern coast of China as well as along the Huai River and Yangtze River floodplains. However, significant overlap in wintering ranges between *C. a. arcticola* and three Siberian subspecies (*C. a. actites*, *C. a. kistchinski*, and *C. a. sakhalina*) has prevented detailed descriptions of wintering distributions. Lanctot *et al*. (2009) used a combination of resighting, molecular and stable isotope techniques in an attempt to identify connectivity patterns between wintering locations and geographically separated breeding grounds for three Dunlin subspecies. Results of this pilot effort were inconclusive but promising, with pending results expected to help resolve differences. The current lack of knowledge about the non-breeding distributions of this species presents a significant obstacle to our understanding of its ecology.

Specifics about the migration routes of *C. a. arcticola* also remain largely unknown, however, the stable isotope data presented in Chapter I supports the presumption that spring migration follows a coastal route (Warnock and Gill 1996). It is

well documented that during the fall migration many *C. a. arcticola* first travel south to the Yukon Delta for refueling prior to departing westward, crossing the Bering Strait to Asia (Taylor *et al*. 2011; Gill *et al.*, in review; R.B. Lanctot, unpubl. data). Location data during spring migration obtained from *C. a. arcticola* tagged with light-intensity geolocator devises suggests a more direct route following the eastern coast of Siberia before crossing at the northern Bering and Chukchi Seas (S. Yezerinac, unpublished data). Migration from Southeast Asia begins in mid-March, peaking in mid-May (Brazil 1991). Arrival at the arctic and subarctic breeding grounds begins in late May, peaking in early June (Holmes 1966a). Once on the breeding grounds, Dunlin form seasonal pair bonds with both sexes sharing in the incubation duties. It is believed that males arrive first to begin territory selection and nest building activities. While courting, pairing and copulation typically occur on the breeding grounds, Holmes (1966a) suggested that in late snowmelt years, pairing might occur prior to arrival. As illustrated in Chapter I, stable isotopes within the tissues of Dunlin may provide some insight into the migratory and breeding behavior of this species.

While stable isotope values of animal tissues can serve as intrinsic chemical markers of past dietary assimilation (Bearhop *et al*. 2004), the metabolic characteristics of various tissues dictate the types of inference that can be made from their isotopic composition. Some tissues are considered metabolically active (e.g. blood, muscle) in that the constituent cells continuously recycle, assimilating new material from the diet (Dalerum and Angerbjörn 2005). These tissues are isotopically dynamic, responding to changes in the isotopic compositions of dietary resources. Other tissues (e.g. feathers, claws) become metabolically inert, or static, after a short period of growth (Mizutani *et*

al. 1990). These isotopically static tissues can be sampled at a time and location that is physically and temporally separated from the where they were grown, providing a snapshot of the isotopic composition of dietary items during the period of growth. Therefore, measuring the isotope ratios of various tissues can provide information about dietary incorporation at different time scales and/or different geographic locations.

In this chapter, I utilized the arrival date information determined in Chapter I in combination with feather isotope data and nesting data to provide new knowledge about Dunlin behavior. As explained in Chapter I, Dunlin transition from a diet presumably based on marine resources during the non-breeding periods to a documented diet based on terrestrial resources on their breeding grounds. I predicted that the substantial 7‰ difference in stable carbon isotopes ($\delta^{13}C$) between marine-based food webs and C_3 terrestrial plant-based food webs (Peterson and Fry 1987) which facilitated my calculations of turnover rates and arrival dates would also allow me to better understand migratory behavior through the isotope values of Dunlin feathers. Additionally, comparing the arrival estimates against individual nesting data provided further knowledge about the behavior of Dunlin on the breeding grounds.

Common among many shorebird species, Dunlin undergo a prenuptial molt of their body feathers prior to or during spring migration, transitioning into their alternate, or breeding, plumage (Holmes 1996b). However, unlike most shorebirds, Dunlin molt most of their primary remiges during incubation on the breeding grounds (Holmes 1966b). Therefore, by capturing Dunlin on their breeding grounds and collecting both body and primary feathers, I was able assess the carbon and nitrogen isotope profiles of

both nonbreeding (wintering or northbound migration sites) and breeding environments. To evaluate differences in feather isotopic composition at the population level, I considered the data in terms of isotopic niche describing the breadth and location of $\delta^{13}C$ - $\delta^{15}N$ space occupied on a bivariate plot (Newsome *et al*. 2007). I assumed that birds from the localized breeding grounds disperse during the fall migration and therefore the body feathers would have been grown across a wider range of wintering and migration locations. Thus, I predicted the stable isotope values of primary feathers would indicate a more constrained isotopic niche than measured in the body feathers as a result of the geographically limited region of the Arctic breeding grounds. Narrowing my scope, I then compared body feather isotope values of paired nesting males and females to assess the potential of shared non-breeding locations. I assumed that individuals utilizing similar non-breeding locations would have more similar isotope values in their body feathers than individuals using more disparate locations. Thus, if male-female pairs persisted through the non-breeding season, it would be detected in the body feather isotopes. Furthermore, as it has been suggested that wintering habitat quality can influence reproduction on the breeding grounds (Marra *et al*. 1998) and because isotope values are reflective of habitat use, I explored for correlations between body feather isotope data and breeding season data to determine if similar effects could be detected in Dunlin.

Whether influenced by wintering habitat or not, evidence suggests that pre-breeding arrival time can have a significant impact on reproductive success (Marra *et al*. 1998, Bearhop *et al*. 2004). Thus, I also explored the relationships of between arrival date estimates and dates of nest initiation at the individual, nesting pair, and population

levels. I predicted the estimated arrival dates would always be earlier than nest initiation dates, since egg formation is thought to occur on the breeding grounds (Holmes 1966a). I also expected the length of time between arrival dates and nest initiation dates would decline as Dunlin arrived later in the summer, assuming late-arriving birds would need to initiate quickly due to the short arctic-breeding season. I also compared the estimated arrival times to the timing of snow melt on the study plots. Here I predicted that arrival would not occur until after the snow melt had begun thus providing sufficient foraging opportunity to replenish energy stores for reproductive and molting activities. By examining the relationships between feather isotopes, arrival times and nesting schedules, I hoped to provide a more comprehensive understanding of Dunlin behavior.

Methods

Study Site Sample collection was conducted during June and July of 2010 and 2011 in the North Slope Borough of Alaska within a 25km radius around the city of Barrow (71°17′44″N, 156°45′59″W; Fig. 1) as described in Chapter I.

Sample Collection In addition to the muscle samples obtained from the collected individuals (described in Chapter I) which were used to estimate arrival dates, I also collected the first primary feather from each wing and six to ten black, breeding plumage contour feathers from the breast or belly region (henceforth, breast feathers) which were then stored at room temperature in paper envelopes.

Two hundred, twenty three nesting adults (103 in 2010, and 120 in 2011) were live-trapped using bow-nets placed over the nest (Bub 1995). All individuals were measured, banded with unique metal and color bands, and sampled for blood and

feathers. I determined sex based on morphological measurements using a discriminant function equation developed for this species (Gates 2011) or through conventional molecular techniques (Griffiths *et al*. 1998). Blood sample collection is described in Chapter I. From each adult, I also collected two primary feathers, one from each wing, and 6-10 breast feathers. If an individual had not begun molting the remiges, I collected the first primary feathers. If the first primaries had been molted, I collected the second primaries. If the second primaries had been dropped, I collected the third primaries; continuing this process through to the seventh primaries. I also collected newly grown first primaries if the feather appeared fully grown and out of sheath. A subset of the captured individuals (n=54) were recaptured during the incubation period to obtain a second blood sample which allowed me to determine the *in-situ* carbon isotope turnover rate as described in Chapter I. At the second capture, if these individuals had fully regrown their first primaries, these feathers were collected to assess isotopic signature changes between years. Thus, in 2010, I collected feathers grown in 2009 and 2010. Feathers collected in 2011 comprise feathers grown in 2010 and 2010 (Table II.1)

All samples were transported to the University of Colorado Denver for storage and tissue preparation. Additional sample preparation and stable isotope analysis was conducted in the laboratories of the USGS.

Isotopic Analysis Isotope values are reported in per mil units (‰) using standard δ notation (Sulzman 2007). Muscle, blood, primary feather, and breast feather samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using an elemental analyzer (Carlo Erba) interfaced to a Micromass Optima mass spectrometer (Fry *et al*. 1992). Procedures for the preparation

and analysis of blood and muscle tissues are described in Chapter I. I washed feathers in a 2 : 1 chloroform and methanol solvent solution to remove surface lipids. Feathers were soaked at room temperature in a series of solvent baths and then allowed to air dry. I analyzed only the barbs of primary feathers by trimming them from the rachis. I removed the filoplume and calamus from the breast feathers but included both the rachis and barbs in the sample. Breast feather samples required one to two feathers from each individual to attain the required sample mass.

Carbon and nitrogen isotopic data were normalized to V-PDB and air using the primary standards USGS 40 and USGS 41. Methods for assessing analytical error, quality control, accuracy, and sample reproducibility (all <0.2‰ across all analytical sequences for both isotopes) are described in Chapter I.

Arrival Date Estimates For this analysis I used the individual arrival date estimates reported in Chapter I determined from whole blood $\delta^{13}C$ values using the *in-situ* carbon isotope turnover rate. While these methods resulted in a distribution of potential arrival dates for each individual, I utilized the median arrival date estimate for each individual in the analyses of this chapter.

Snowmelt Progression I estimated percent snow cover through visual observations of study plots from late May to the middle of June each year according to standardized protocols (Arctic Shorebird Demographic Network Protocol Subcommittee 2010) as described in Chapter I.

Nest Initiation Several methods were used to determine nest initiation dates (i.e., date the first egg is laid) for captured birds. For nests found with incomplete clutches (<4

eggs), I counted back one day for each egg present to estimate the nest initiation date. If clutches were never completed or if the nest was found with a complete clutch of eggs, I floated at least two eggs to estimate the start of incubation and then back-calculated an additional day for each egg in the nest (Liebezeit *et al.* 2007). If nests were found when eggs had begun hatching (visible cracks or holes), I did not float eggs but observed the nest daily until chicks were observed. Then, nest initiation was back calculated from the hatch date using a mean incubation period for Dunlin of 21 days (Holmes 1966a) plus one day for each egg laid. Because both males and females are required for egg fertilization and Dunlin form seasonal pair bonds with both sexes sharing incubation duties, the nest initiation date represents the latest possible date of arrival for either individual in the nesting pair.

Statistical Analysis I used a Pearson's product-moment correlation test for comparisons between daily average percent snow-cover and the daily cumulative number of captured birds present, snow-cover and the cumulative number of nests initiated, individual breast feather $\delta^{13}C$ and $\delta^{15}N$ values, breast feather isotope values of breast feathers of nesting pairs, arrival date estimates and nest initiation dates. I used a permutation analysis to test for annual variation in arrival date estimates, nest initiation dates, and lag times between arrival and nest initiation. When a difference was found, I used a Wilcoxon Rank Sum test to determine directionality. I tested for inter-annual differences in mean $\delta^{13}C$ and $\delta^{15}N$ values for breast feathers using Welsh's t-tests. I tested for differences in primaries first by feather group (grown in 2009 but collected in 2010, grown and collected in 2010, grown in 2010 but collected in 2011, and grown and collected in 2011) and then by feather number (1-7) using a one-way ANOVA followed

by a Tukey's HSD pairwise comparison. To compare the isotopic niche breadths of wintering and breeding periods, I used the SIBER (Stable Isotope Bayesian Ellipses in R; Jackson *et al*., 2011) method in the SIAR package (version 4.1.3) to measure the standard ellipse areas (SEA_B) in $\delta^{13}C$-$\delta^{15}N$ space for breast feathers and primary feathers. This method uses a Bayesian approach to integrate differences in sample size and other sampling uncertainties. All analyses were conducted in the R statistical computing package (version 3.0.0; R Development Core Team, 2013).

Results

Of the 223 individuals trapped over 2010 and 2011, 28 were trapped both years. The sex ratio of captured adults was not different from 1:1 (Binomial test, 2010: $p = 0.84$; 2011: $p = 0.65$).

Breast Feathers The $\delta^{13}C$ and $\delta^{15}N$ values of breast feathers collected in 2010 were not statistically different from those collected in 2011 ($\delta^{13}C$: $t = 0.7$, $df = 229.3$, $p = 0.45$; $\delta^{15}N$: $t = 0.8$, $df = 236.9$, $p = 0.41$). Mean $\delta^{13}C$ and $\delta^{15}N$ values of all breast feathers were -14.0‰ (±2.4SD) and 14.1‰ (±2.2SD), respectively (Fig. II.2). For individuals captured in both 2010 and 2011, differences in $\delta^{13}C$ and $\delta^{15}N$ values of breast feathers collected in each year were not statistically significant (paired Wilcoxon test: $\delta^{13}C$: $V = 234$, $p = 0.49$; $\delta^{15}N$: $V = 202$, $p = 0.76$). Breast feather $\delta^{13}C$ and $\delta^{15}N$ values of male-female nesting pairs did not show any significant correlation (for both $\delta^{13}C$ and $\delta^{15}N$: $\rho = 0.03$, $p > 0.82$).

Primary Feathers Pooling all primary feathers, the mean $\delta^{13}C$ and $\delta^{15}N$ values for the population were -25.2‰ (±1.24 SD) and 9.0‰ (±0.86 SD), respectively (Table

II.2; Fig. II.2). I initially split the primary feathers into four groups for analysis: (A) primaries grown in 2009 but collected in 2010, (B) primaries grown and collected in 2010, (C) primaries grown in 2010 but collected in 2011, and (D) primaries grown and collected in 2011. Differences in mean $\delta^{15}N$ values between these groups were not statistically significant (one-way ANOVA: $F = 0.26$, $p = 0.86$). Mean $\delta^{13}C$ values of feathers grown in the year prior to collection (groups A and C) were lower than feathers grown in the same year they were collected (groups B and D) (Wilcoxon: $W = 3053.5$, $p < 0.001$). I suspect that this difference may be due to the type of primary feathers included in these groups. Whereas the groups B and D were comprised of mostly first primaries (n=67 of 69 total), groups A and C (n=190) were comprised of first through seventh primaries. Because Dunlin molt their primary feathers sequentially (Holmes 1966c), the seventh primaries are grown much later in the summer than the first primaries. As demonstrated in Chapter I, blood tissues transition from higher to lower $\delta^{13}C$ values post-migration as their tissue isotope values equilibrate with the local diet. Thus, it is likely the primaries grown early in the season are being produced, in part, from resources obtained during migration with higher $\delta^{13}C$ values. When I group feathers by primary number (1-7) this trend is fairly clear, with feathers grown later in the season having lower $\delta^{13}C$ values on average (Fig. II.3). If I assume the sixth and seventh primaries had the least input from the migration diet, mean $\delta^{13}C$ and $\delta^{15}N$ values of those feathers, grown mainly on terrestrial resources, were -26.0‰ (±1.12 SD) and 9.0‰ (±0.88 SD), respectively. The difference in mean $\delta^{13}C$ value between these outer primaries and the muscle samples of post-breeding birds (-26.2‰; SD ±1.0; see Chapter I) was not statistically significant ($t = -0.3673$, $df = 11.57$, $p = 0.72$). However, the

difference in mean $\delta^{15}N$ values between the outer primaries and muscle samples of post-breeding birds was statistically significant ($t = -2.3$, $df = 10$, $p = 0.04$).

Within individuals that were captured in both 2010 and 2011, the mean differences in primary feather $\delta^{13}C$ and $\delta^{15}N$ values between years were not significant (paired Wilcoxon test: $V = 79$, $p = 0.21$; $V = 102.5$, $p = 0.45$, respectively) even though different primaries (e.g. first and fifth) were sampled in different years for some birds.

Isotopic Niche Breadths There is a clear separation in the $\delta^{13}C$ - $\delta^{15}N$ space occupied between breast feathers and primary feathers (Fig. II.2). The Bayesian approach allows me to compare the total area of probability distributions in $\delta^{13}C$-$\delta^{15}N$ space (in $‰^2$; Fig. II.3). In 2010, the results indicate that the isotopic niche breadth of breast feathers (mean = $17.5‰^2$) was approximately 4.6 times larger than measured in the primary feathers (mean = $3.81‰^2$). In 2011, breast feather niche breadth (mean = $16.6‰^2$) was approximately 5.7 time larger than primary feather niche breadth (mean = $2.9‰^2$). Niche breadths of pooled 6^{th} and 7^{th} primaries did not differ from the larger sample of primaries in each year (2010: $t = -398$, $df = 1632538$, $p < 0.001$; 2011: $t = -355$, $df = 1728205$, $p < 0.001$).

Arrival Date Estimates The estimated arrival dates for the 2010 population ranged from 21 May to 15 June with a median arrival date of 6 June (Fig. II.1). In 2011, the estimated arrival dates ranged from 24 May to 22 June, with a median estimated arrival date of 9 June. The median arrival date in 2010 was significantly earlier than in 2011 ($Z = -5.0$, $p < 0.001$; one-tailed Wilcoxon Rank Sum: $W = 3888.5$, $p < 0.001$).

For both years, arrival dates estimates showed a strong negative correlation with the progression of snowmelt (2010: $\rho = -0.82$, 2011: $\rho = -0.97$; $p < 0.001$). At the population level, my analysis indicates that there is no difference between male and female median arrival dates in either year ($W = 6600$, $p = 0.41$). However, in nesting pairs (n=95) where both individuals were sampled, males did have a slight tendency to have arrived before the females (paired Wilcoxon Rank Sum: $V = 1493$, $p = 0.05$). The median difference between nesting-pair male and female arrival dates was -1 day (±4.4 MAD). Arrival date estimates showed only a weak correlation with breast feather carbon isotope values ($\delta^{13}C$: $\rho = 0.13$, $p = 0.058$; $\delta^{15}N$: $\rho = -0.001$, $p = 0.99$).

Nest Initiation Dates Nest initiation dates did not differ significantly between 2010 and 2011 ($Z = -0.3341$, $p = 0.75$) with a median nest initiation date of 13 June (range: June 5 – June 26; Fig. II.1). However, individual lag-time between median arrival date estimates and nest initiation dates did differ between years. In 2010, the median individual lag-time was 7 days (± 5.9 MAD) as compared to 4 days (± 4.4 MAD) in 2011. Male and female lag times did not differ ($Z = -1.267$, $p = 0.21$). Nest initiations displayed a strong negative correlation with snowmelt in both years (2010: $\rho = -0.89$, 2011: $\rho = -0.97$; $p < 0.001$). Nest initiation dates presented essentially no correlation with breast feather isotope values ($\delta^{13}C$: $\rho = 0.03$, $p = 0.67$; $\delta^{15}N$: $\rho = 0.01$, $p = 0.84$).

Discussion

Although both years showed a strong correlation between the progression of snowmelt and the cumulative number of Dunlin onsite (Fig. II.1), there was an apparent disparity among years. In 2010, the majority of snowmelt occurred approximately two

weeks later in the season than in 2011; whereas the 2010 mean arrival date was three days earlier than in 2011. The fact that a dramatic shift in snowmelt date had little effect on arrival dates suggests that other mechanisms are responsible for mediating arrival date. It is likely that departure from the wintering grounds is timed by changes in photoperiod (Gwinner & Brandstätter 2001) which in turn, assuming a standard travel time, would regulate arrival times. Based on a theory first proposed by Lack (1954) it follows that arrival times are targeted to allow sufficient time for courting and incubation such that hatch occurs during the peak abundance of food sources. Holmes (1966a) demonstrated that Dunlin hatch does coincide with the peak emergence of invertebrate prey items and Hill (2012) found that chick survival is maximized when hatch occurs at or just prior to peak emergence providing strong evidence in support of Lack's theory. While I had hoped to demonstrate a relationship between breast isotope values and breeding behaviors (i.e. arrival dates and nest initiations), the data does not show any significant relationships. This does not mean that wintering habitat does not affect reproduction; it simply shows that feather isotopes are not suitable for addressing this issue in this species.

However, carbon and nitrogen isotope values measured in the feathers still provide useful information about the distribution and ecology of Dunlin. Sampling both breast and primary feathers allowed me to evaluate the isotopic niche of Dunlin at two temporally and geographically distant locations. Comparing the two feather types, I found that not only are the mean $\delta^{13}C$ and $\delta^{15}N$ values of breast feathers significantly higher than for primary feathers, but also the isotopic niche breadth of breast feathers is much larger than for the primaries.

In general, primary feather $\delta^{13}C$ and $\delta^{15}N$ values indicated a diet of distinctly terrestrial food sources. The decrease in $\delta^{13}C$ values as feathers are grown further in time from their arrival date is consistent with the transition of $\delta^{13}C$ that I measured in the blood tissues. Thus, it is likely resources obtained during migration contribute to the production of the innermost primaries. The isotope values of the six and seventh primaries, therefore, most accurately reflect the isotope values of feathers produced from the terrestrial prey available around Barrow. Comparing the $\delta^{13}C$ values of these outer primaries to a mean value of -27.3‰ from published values of prey items collected at this location (Yohannes *et al*. 2010, Oppel and Powell 2010) suggests a diet-feather discrimination factor of approximately 0.8‰. The similarity between $\delta^{13}C$ values of these outer primaries and muscle samples from post-breeding birds suggests that 6th and 7th primary feathers are grown primarily from exogenous resources obtained in the terrestrial breeding grounds.

The range of $\delta^{13}C$ and $\delta^{15}N$ values for breast feathers grown in the non-breeding season was both higher and broader than expected for a presumed marine-based diet. One possible explanation is that they were feeding in coastal habitats containing seagrasses. Short *et al*. (2007) have reported a wide distribution of seagrasses growing in the coastal areas of Southeast Asia with moderate to high levels of seagrass diversity in this region. High $\delta^{13}C$ isotope values, ranging from -15.5‰ to -5.6‰, have been reported for multiple species of seagrass (Andrews and Abel 1979). More recent studies in the French Atlantic and the Florida coast (Anderson and Fourqurean 2003, Lebreton *et al.* 2012) reported $\delta^{13}C$ values for seagrass in the range of -11.1‰ to -7.2‰. Seagrasses can support high levels of biofilm production (Merina *et al.* 2011). Hladyz *et al.* (2011)

demonstrated that biofilms primarily use the substrata (e.g. seagrass leaves) as a carbon source. Biofilms, in turn, represent a primary dietary resource for marine invertebrates (Thompson *et al*. 2000) which are the assumed primary diet source for wintering Dunlin. Therefore, assuming Dunlin are feeding in areas proximate to seagrass habitat, the high $\delta^{13}C$ values in Dunlin breast feathers are likely a result of the high seagrass $\delta^{13}C$ values. Additionally, morphological structures on the tongue and biofilm presence in stomachs of Dunlin indicate active biofilm feeding (Elner *et al.* 2004, Mathot *et al.* 2010) providing a more direct route to accessing seagrass carbon.

$\delta^{15}N$ values in breast feathers were also relatively high, which may also be related to feeding in habitats associated with seagrass production. Lebreton *et al.* (2012) reported seagrass $\delta^{15}N$ values ranging from 4.9‰ to 9.9‰. Adding a 3-4‰ increase in $\delta^{15}N$ values with each trophic level from seagrass to Dunlin would place these values within the range of $\delta^{15}N$ values measured in the feather tissues. However, Hladyz *et al.* 2011 suggest that, at least when the substrate is nitrogen limited, some bacterial species in biofilm may rely on dissolved nitrogen available in the water. This is likely to contribute to the variability of $\delta^{15}N$ values seen in the Dunlin breast feathers.

The larger isotopic niche breadths measured in the breast feathers suggests a much higher level of isotopic heterogeneity in the dietary resources consumed in the wintering habitat or along the migration route. Differences in prey items between the coastal non-breeding environment and the terrestrial breeding environment are to be expected. However, a critical component to be considered when interpreting the difference in niche breadths is the geography of where these feathers were grown. All

Dunlin were captured within a 25 kilometer radius around the city of Barrow, a relatively small area. Based on Holmes' (1966b) study on dunlin molting patterns and my own field assessments of molt progression, I can safely assume that the primary feathers were grown on the breeding grounds. The terrestrial isotope signatures measured in these feathers supports this assumption. In contrast, breast feather molt may occur anywhere throughout the wintering range (>20 degrees of latitude; Warnock and Gill 1996) as well as during northbound migration (Choi *et al*. 2011). The inverse relationship between $\delta^{13}C$ values and latitude that has been reported in both marine and terrestrial ecosystems is likely to contribute to the isotopic variability measured in the breast feathers; similar relationships have been shown in $\delta^{15}N$ values of marine mammals and seabirds (see Kelly 2000). Because I do not know where individuals molted their breast feathers, the larger niche breadth observed in the breast feathers is likely reflective of the extensive geographic distribution of these Dunlin during the prenuptial molt. Anthropogenic activities in this rapidly developing part of the world are also likely to have influence on the variability of stable isotope ratios in Dunlin feathers (Hobson 1999).

In addition to the non-breeding information provided by the feather isotope values, the relationships between arrival, nest initiation and snowmelt data provide a better understanding of Dunlin behaviors on the breeding grounds. The similarity of male and female arrival dates, both at the population level and within nesting pairs, appears to contradict the belief that males arrive early for territory selection and defense. While median arrival dates differed significantly between years, the median nest initiation date remained essentially the same. Considering the difference in conditions between years, with 2010 being a late snowmelt year, the static nest initiation date

indicates an optimal laying date which may be less influenced by local conditions early in the breeding period, but rather targeted at timing hatching dates for optimal rearing success. The longer lag-times in 2010 between arrival and nest initiation may be the result of individuals delaying nesting activities until sufficient territories became snow-free; with territories being claimed and nests initiation as rapidly as they come available. It may also result from a higher proportion of individuals laying an early nest which was never detected, losing it due to the unfavorable conditions and then initiating a second clutch. In 2011, with most of the tundra snow-free and available for nesting prior to the peak arrival dates, it appears that Dunlin spend little time selecting territories and mates and get right to the business of procreation.

Conclusion

Like many migratory avian species, Dunlin lie below the threshold of size requirements for carrying traditional, external devices capable of long-term and long-distance tracking (e.g. GPS). Thus, ascertaining migratory information about this species can be a difficult and costly endeavor. The stable isotope techniques used in this study served as a relatively simple method for providing novel insight into the migratory behaviors of Dunlin. The isotopic composition of Dunlin breast feathers indicates a more diverse habitat use in the non-breeding season than previously thought. Carbon isotope values measured in the primary feathers suggest that endogenous resources obtained during migration are invested in the initial stages of the post-nuptial molt that occurs on the breeding grounds. The post-migration changes in blood isotope values which allowed me to calculate individual arrival times has helped improve our understanding of the

dynamics of Dunlin breeding biology and how it may be influenced by changing environmental factors.

Historically, animal ecologists have often been limited to considering these types of migratory and breeding behaviors at the population level. The application of these isotopic techniques, in conjunction with other rapidly developing monitoring technologies, will allow me to better understand how individual behavior drives population level processes. These techniques are widely applicable across a diversity of taxa, geographies, and environments. They have and will continue to provide fundamental information for improving the conservation and management of wild species and will provide vital knowledge about the influence of climate change on migratory species. In this time of rapid environmental change, we need accurate and effective tools to monitor the transformations taking place in our ecosystems; it is only through informed decision-making that will allow us to successfully adapt to our future.

Tables and Figures

Table II.2. Numbers of individuals with primary feathers collected

Feather counts are separated by year grown and year collected.

	Individuals with **only 2009** primaries collected	Individuals with both **2009 and 2010** primaries collected	Individuals with **only 2010** primaries collected	Individuals with both **2010 and 2011** primaries collected	Individuals with **only 2011** primaries collected	**Total**
Collected in 2010	56	36	15	-	-	**107**
Collected in 2011	-	-	95	13	15	**123**
Total	**56**	**36**	**110**	**13**	**15**	**230**

Table II.3. Primary feather isotope values ($\delta^{13}C$ and $\delta^{15}N$)

Mean $\delta^{13}C$ and $\delta^{15}N$ values (units in ‰) of primary feathers (standard deviations in parenthesis); separated by year grown and year collected. Groups within each row that had significantly different isotope values are indicated by different superscripts.

	Collected in 2010		Collected in 2011		
	Primaries grown in 2009	**Primaries grown in 2010**	**Primaries grown in 2010**	**Primaries grown in 2011**	**All Primaries (2009-2011)**
$\delta^{13}C$	-25.5^{a} (1.34)	-24.6^{b} (0.88)	-25.5^{a} (1.20)	-24.4^{b} (0.73)	-25.2 (1.24)
$\delta^{15}N$	9.0^{c} (0.97)	9.1^{c} (0.91)	9.0^{c} (0.75)	9.1^{c} (0.81)	9.0 (0.86)

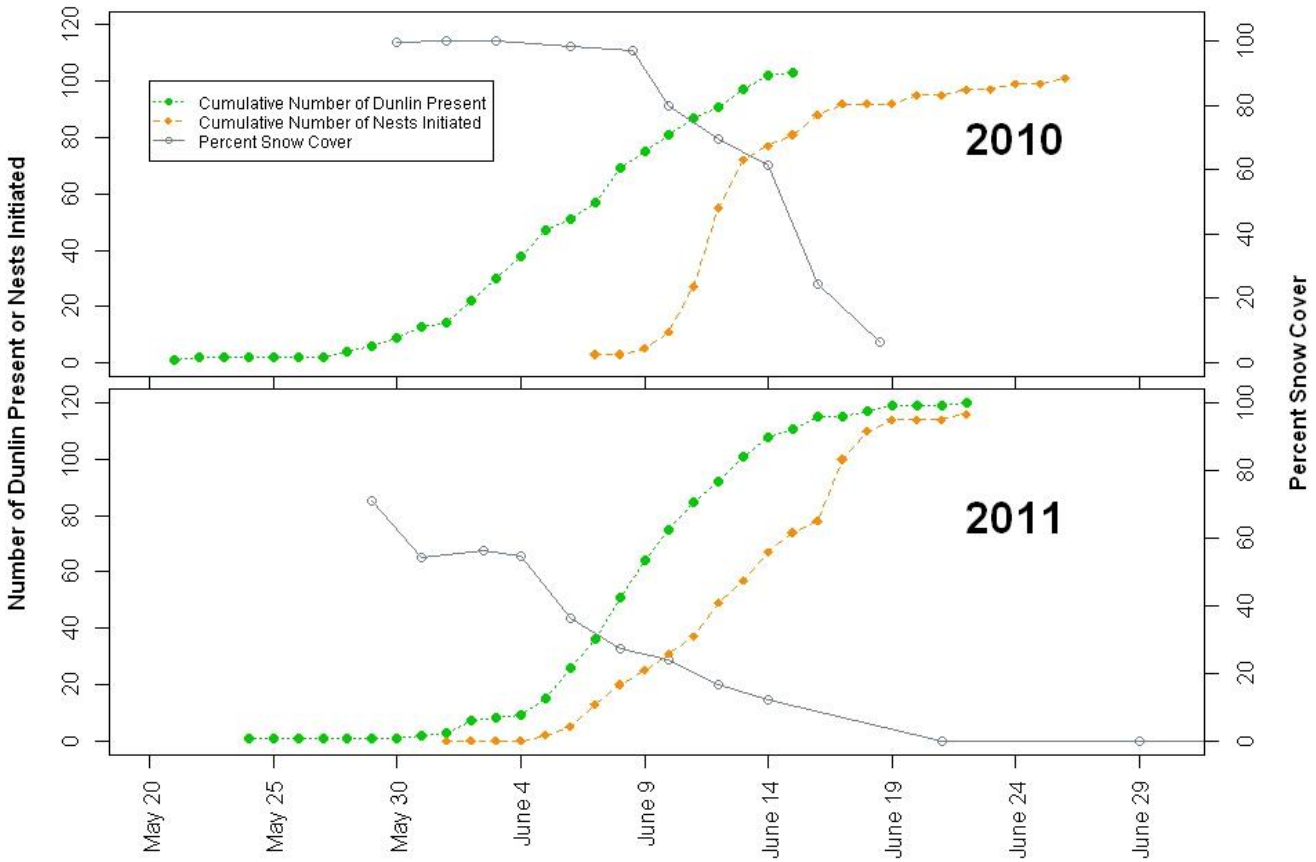

Figure 0.1 Snowmelt, Dunlin arrivals and nest initiations

Progression of snowmelt, Dunlin arrival estimates and nest initiations for 2010 and 2011. Dunlin arrival estimates are combined to show the cumulative number of Dunlin present (solid green circles). Percent snow cover is represented by the open black circles. The cumulative number of nests initiated is represented by solid orange circles.

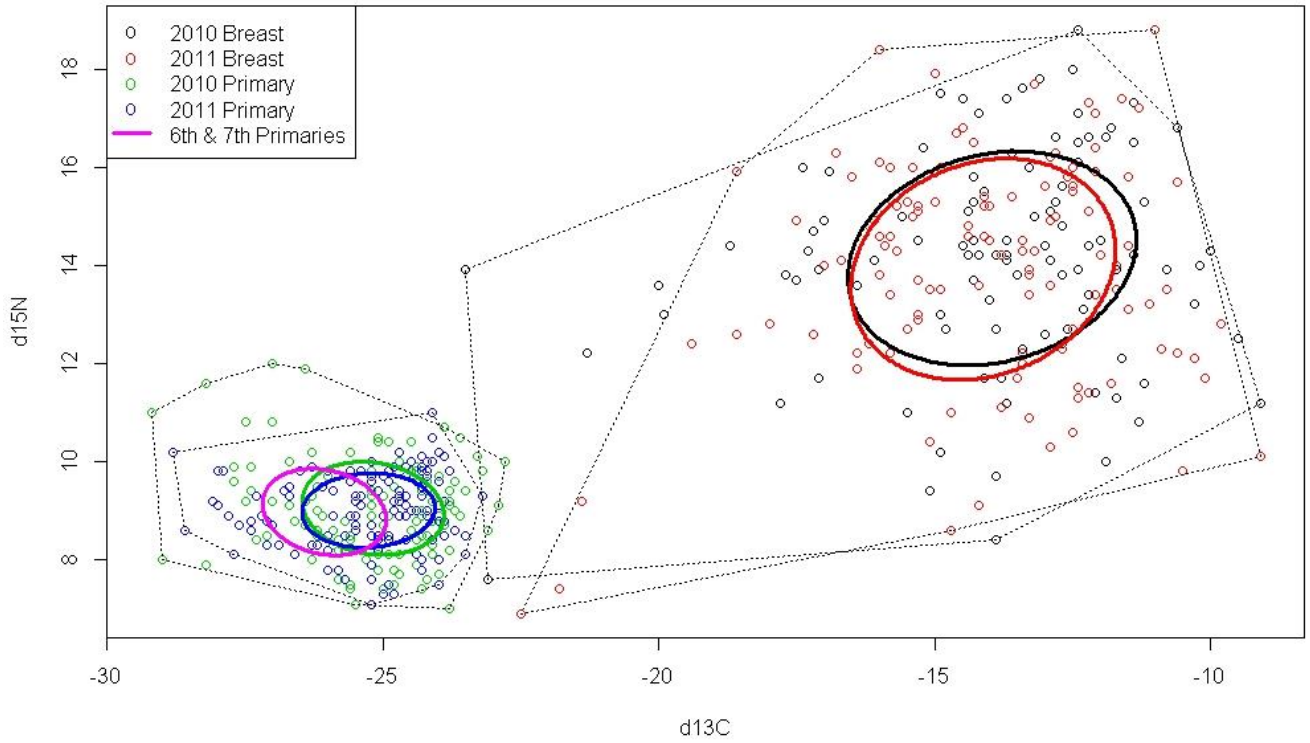

Figure 0.2 Dunlin feather isotopic niches ($\delta^{13}C$ and $\delta^{15}N$)

A bivariate plot displaying the $\delta^{13}C$ and $\delta^{15}N$ values of Dunlin feathers collected in Barrow, AK. Circles represent individual values from breast feathers (black=collected in 2010, red=collected in 2011) and primary feathers (green=collected in 2010, blue=collected in 2011). Ellipses contain approximately 40% of the isotope values from each feather group of corresponding color. The dotted lines denote the convex hull containing all values of each group. The magenta ellipse represents a 95% confidence interval for 6th and 7th primary feathers collected in both years.

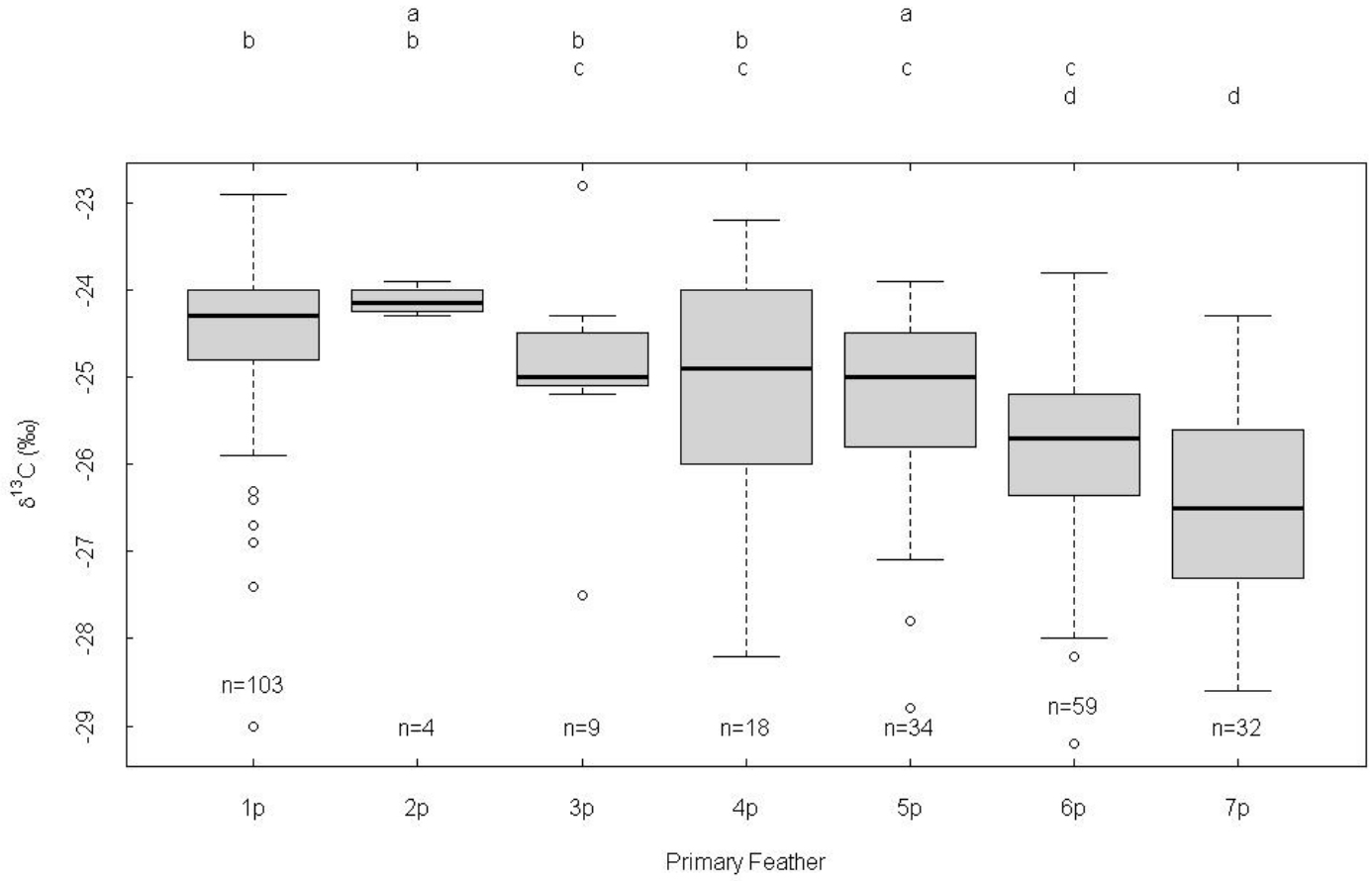

Figure 0.3 Primary feather isotope values

Distributions of $\delta^{13}C$ values of primary feathers separated by feather number (e.g. 1p=first primaries, 2p=second primaries, etc.). Sample sizes are shown below each boxplot. Letters above plot indicate groups of feather numbers in which $\delta^{13}C$ values are not statistically different (e.g. 6p values are not statistically different than 7p values).

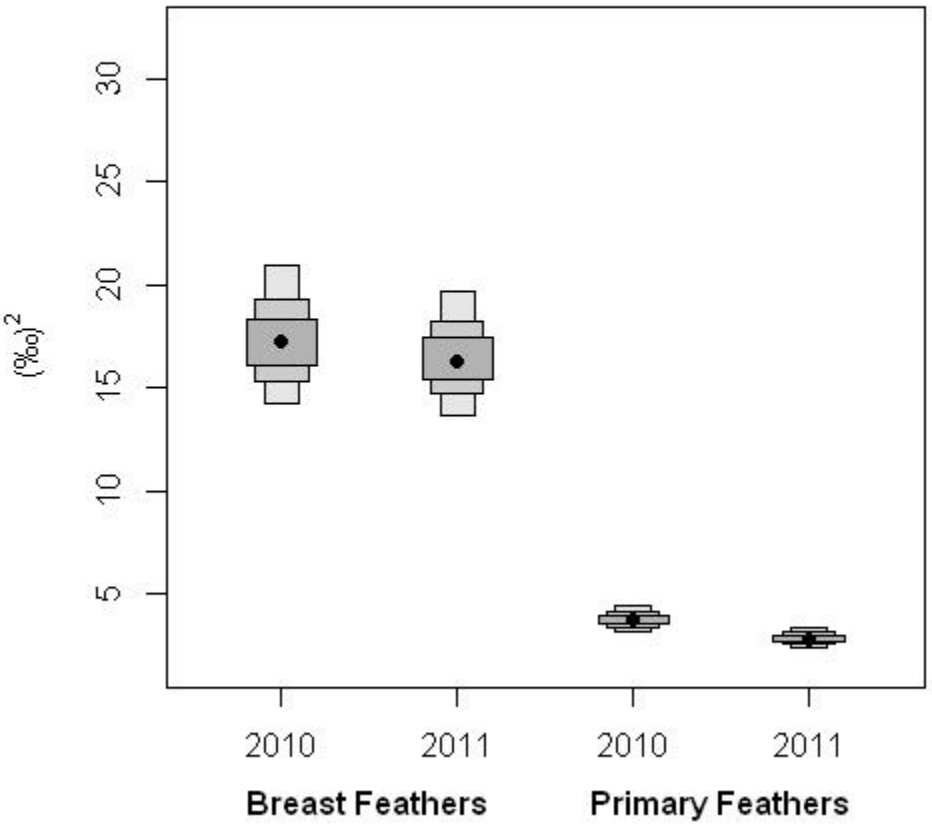

Figure 0.4 Standard ellipse areas of Dunlin feather isotopic niches

Probability distributions of the standard ellipse area, or SEA_B (in $‰^2$), for breast feathers and primary feathers collected in 2010 and 2011. Boxes represent the 50%, 75% and 95% credible intervals for each group; dots represent the group mode.

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